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"PERFUSION - PRESERVATION OF SMALL INTESTINE"

BY

© ALLEN E. FRISSELL

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF M.Sc.

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Perfusion - Preservation of Small Intestine", submitted by Allen E. Frissell in partial fulfilment of the requirements for the degree of M.sc. in Experimental Surgery.

ABSTRACT

Experiments have been performed to establish the effectiveness of hypothermic perfusion-preservation on segments of terminal ileum. The segments were perfused with cryoprecipitated plasma at a temperature of 10°C. Segments perfused for four, eight and 24 hours were observed for gross changes, and after various intervals biopsies were taken for histologic examination. Vitamin B_{12} absorption from autografted, preserved segments was determined. The use of an oxygenator and the addition of chlorpromazine to the perfusate gave better preservation. Vascular thrombosis occurring early post autografting was a major problem. It was in part corrected by premedicating the experimental animals with acetylsalicylic acid, prednisone and dipyridamole; and by bathing the vascular anastomosis with a dilute solution of magnesium sulfate.

Using these techniques, it was finally possible to consistently preserve segments for eight hours, and have them absorb Vitamin B_{12} after autografting. A variable degree of mucosal slough occured early in all segments of preserved intestine. Those segments preserved for eight hours or less were capable of regenerating a normal mucosa from the cells remaining in the basal crypts. Segments preserved for 24 hours showed some signs of viability, however, they did not survive more than 48 hours after re-implantation.

The results of the study are encouraging for they demonstrate the ability of the intestine to withstand the rigors of hypothermic perfusion, and suggest that preservation for longer periods is possible with improvements in perfusion techniques.

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I. Introduction.

A. Importance of Intestinal Transplantation and Preservation.

It is estimated that several thousand patients die each year in the United States lacking only the few feet of small intestine required for survival (1). These patients have lost most of their small intestine because of mesenteric vascular thrombosis, strangulation obstruction, congenital malformations, regional enteritis, abdominal trauma, or extensive neoplasm (2). Death or chronic invalidism is the prospect facing these patients. Life may be prolonged by means of intensive intravenous feeding, but intestinal transplantation offers the only possibility of long term survival.

The intestinal rejection phenomenon, and immunosuppression to extend the life of the transplanted intestine has been investigated in both laboratory and clinical situations (1,2). Experiments in this laboratory have achieved prolonged survival of intestinal allografts in dogs, the animals maintaining normal nutrition and weight on a regular diet for up to a year and a half (3).

It seems likely that intestinal transplantation may soon become a feasible clinical technique, as renal transplantation is today. The renal experience has shown that preservation of the organ to be transplanted is often necessary if wastage and loss of grafts is to be avoided. Therefore, we have undertaken a series of experiments to establish the feasibility of intestinal preser-

vation using a hypothermic perfusion technique.

B. Importance of Organ Preservation.

Current interest in organ preservation has been stimulated by advances made in clinical organ transplantation during the last decade. The early success of renal transplantation created a demand for viable kidneys that could only be met by a living donor or fresh cadaver. The development of practical preservation techniques for the kidney have helped to diminish the air of emergency that had previously been part of clinical transplantation. Organ preservation allows sufficient time for histocompatability testing, preparation of the recipient, and even for transportation of the organ to a distant hospital (4,5,6).

C. Choice of Preservation Method.

Methods of organ preservation have developed slowly over the past century. At present, two basic techniques are used: complete support of the organ metabolism, or support of a reduced organ metabolism. Theoretically, the second of these methods is the best. It offers simplicity and the possibility that the organ may be preserved for extended periods. Unfortunately, none of the possible methods of organ preservation has been perfected.

In selecting a preservation method for intestine, perfusion and hypothermia seemed most applicable (4,5,7,8). At this time, kidneys, livers, and hearts are best preserved by methods involving perfusion. The best method for preserving organs varies with

each organ type: kidneys, by hypothermic perfusion (9): livers, by hyperbaric oxygenation and hypothermic perfusion (10): and hearts, by normothermic perfusion (11). Because most organs have been successfully preserved with a combination of hypothermia and perfusion, these two modalities were used in the present study of intestinal preservation.

There are several reports describing the perfusion of intestine, and also the use of hyperbaria and hypothermia in intestinal preservation. There is only one report (unsuccessful) in which perfusion and hypothermia were combined for intestinal preservation. See appendices one and two.

2. Methods of Preservation.

A. Hypothermia.

i. Useful Effects of Hypothermia.

Hypothermia is used in organ preservation because it lowers metabolic activity, decreasing nutritional and respiratory needs (4,5,12). In perfused kidneys and other organs, an exponential decrement in metabolic activity with decreasing temperature has been demonstrated (13,14).

Although oxygen requirements diminish with hypothermia, a uniform depression of all metabolic activity does not occur, since each enzyme system has a unique Q_{10}. For example, renal amino acid absorption is normal at 5°C (14). Therefore, it is often necessary to support cellular processes which are not completely inactivated by hypothermia.

Hypothermia aids in the oxygenation of a perfused organ. The oxygen content of a solution lacking hemoglobin is a function of the partial pressure of the gas and its solubility co-efficient. The solubility co-efficient increases with reduction in temperature, thus increased amounts of oxygen will physically dissolve in aqueous solutions (15). With temperatures of 10°C or less, the partial pressure of oxygen may be easily increased with an oxygenator so that low flow rates and physiological perfusion pressures may be used to provide a cold organ with sufficient oxygen (4).

In perfusion systems, the rate of perfusate flow is markedly affected by hypothermia. This is the result of temperature effects on vasomotion. The complete effect of hypothermia on the vascular resistance of an isolated organ cannot be fully evaluated since denervation, vasoactive substances, tissue pressure and vasomotion from other causes will alter any single effect due to hypothermia (16,17,18,19,20). However, after an initial brief period of vasoconstriction with the onset of hypothermia, the perfusion pressure usually drops to slightly below normal due to hypothermic vasomotor paralysis (4,7,21,22,23). This allows lower perfusion pressures to produce an effective flow rate.

Bacterial growth may be a problem in long term perfusions, and has been reported as a complication of a six day hypothermic renal perfusion (24). However, because most bacteria inhabiting mammals survive optimally at temperatures between 40°C and 20°C, their growth rate and potential for inflicting damage are reduced at hypothermic temperatures (12).

ii. Harmful Effects of Hypothermia.

In addition to the beneficial effects of hypothermia on organ preservation, low temperatures are also associated with problems. Cellular edema is invariably observed with hypothermia unless the organ is otherwise protected (17,22,24,25,26). The edema is the result of sodium pump depression which disrupts the normal transmembrane equilibrium of sodium, potassium and water. Accumulation of toxic byproducts can result in cellular damage due to the inactivation of essential enzymes in metabolic chain reactions (27). Plasma proteins are denatured by hypothermia, and damage the organ when they precipitate and embolize to the peripheral capillary beds (28). At hypothermic temperatures, hemoglobin is a less effective buffer and carrier of carbon dioxide (29,30). Oxygen is bound more firmly to hemoglobin, interfering with oxygen release at the tissue level. Increased fluid viscosity at low temperatures is not always compensated by hypothermic vasodilation, and therefore physiological flow rates and perfusion pressures may be difficult to attain (4,31).

Nerve and muscle activities are lost at low temperatures. Depression of conduction and contractibility is apparent at about 20°C, and full inhibition occurs between 10°C and 8°C (32).

Viability for most organs with hypothermic preservation alone is limited to less than twelve hours (5). For the intestine, it is only five hours (33).

B. Perfusion.

i. History of Organ Perfusion.

The kidney was the first organ (1849) to be perfused Ex Vivo (34). The first successful perfusion of intestine (dog and rabbit), was done by Salvioli (1880). He used defibrinated blood diluted in saline as the perfusate (35).

A practical perfusion unit was designed for the heart in 1895 by Landendorff. Non-pulsatile gravity flow was employed which did not recirculate the electrolyte perfusate (36). A technique of autoperfusion of the heart was described in 1920 by Knowlton and Starling (37). Later famous as the "Starling Preparation", it was used to perfuse and study the function of the isolated kidney (38). The development of the Lindbergh-Rockefeller Institute organ perfusion system resulted in the culture of thyroid, ovaries, hearts, kidneys and pancreas. Artificial culture medium was propelled from a reservoir to the isolated organ by pulsatile gas pressure. A controlled, sterile environment was an important factor in the success of this experiment (39).

The introduction of heparin anticoagulation permitted the use of whole blood in isolated organ perfusion (34). It made possible the use of a rotating cylinder oxygenator as the first heart and lung replacement in experimental animal perfusion (40).

The current useful principles of isolated organ perfusion-preservation may be gleaned from published data derived from

extensive work done on the kidney, heart, liver and lung during the past decade. Humphries has reviewed the problems of organ perfusion. These he lists as tissue edema, vasoconstriction, capillary obstruction, traumatic apparatus, maintenance of nutrient supply, and inhibition of bacterial growth(7).

ii. Introduction.

Artificial perfusion closely simulates normal blood circulation, and is probably the most physiological method for the maintenance of In Vitro organs. It seems to offer the best prospect of prolonged organ preservation.

Perfusion-Preservation of organs has been undertaken in several ways: Ex-Vivo perfusion, intermediate host storage, and by mechanical devices, employing pumps and oxygenators.

Ex-Vivo perfusion involves the connection of the isolated organ to a support animal by means of direct arterial and venous shunts. The support animal may be the donor (41). If another support animal is used for long term preservation, problems associated with allografting must be dealt with. Extended preservation of kidneys and hearts have been successful with appropriate immunosuppression (42,43).

Intermediate host storage involves the temporary transplantation of an organ into an intermediate host. One example is the storage of human kidneys in baboons (44). This is a physiologic expedient in that the artificial components and support measures of a perfusion system are avoided. However, host immunosuppression,

especially where organs have been placed in other species, has often failed.

An isolated organ is at present most conveniently maintained by means of a heart-lung machine. A pump for perfusion, an oxygenator for gaseous exchange, a perfusate, nutrient supplements and pharmacologic agents form the armamentarium.

The techniques and applications of perfusion are complicated. Simply stated, the demands of the organ must be met by a specifically defined medium which is to be delivered to the organ in a way that certain physical requirements are met (1). These are summarized in table I.

TABLE I.

PRINCIPLES OF ORGAN PRESERVATION

1. Requirements:
   - Oxygen
   - Nutrients
   - CO_2 and Waste Removal
   - Electrolytes
2. Delivery:
   - Inflow and Outflow Pressure
   - Flow Rate
   - Flow Quality
   - Conductance
   - Compliance
3. Perfusate:
   - Derivation
   - Viscosity
   - Osmotic and Oncotic Pressure

iii. Oxygen Supply.

Delivery of oxygen depends on perfusate flow rate, oxygen carriers in the perfusate, and the perfusate partial pressure of oxygen. Ignoring the ability to adjust flow rate, the most important technical advance has been the oxygenator because it permits ready manipulation of the perfusate PO_2 (34,35). Temperature is important since hypothermia increases the solubility co-efficient of oxygen in aqueous solutions (15). Oxygen transport has been experimentally improved with various synthetic oxygen binders such as silicone, fluorocarbon, and plant oil emulsions used to replace hemoglobin in perfusates (46).

The oxygen requirements of isolated perfused organs might be estimated by extrapolating normothermic oxygen uptake to hypothermic conditions (4). However, in practice, it has been best to find the requirements empirically. Belzer has answered this question for the kidney in finding optimal preservation with a venous PO_2 of over 80 mmHg. (23). Arterial PO_2's as low as 89 mmHg. have been used in renal perfusions, and under the experimental conditions, not all of the available oxygen was extracted (47). The optimal perfusate oxygen content for intestinal preservation is unknown.

Normal intestinal oxygen consumption varies according to the metabolic activity of the bowel. Normal values are reported in several papers:

Consumption (cc/gm/min)	Reference
0.004 to 0.041	52
0.0075	101
0.09 (dry weight)	76

Theoretically, the oxygen consumption of intestine at 10°C is about 0.001 cc/gm/min (144).

iv. Oxygen Toxicity.

The correct amount of oxygen to be delivered to a perfused organ is not clearly defined (4,7). It must be high enough to support the metabolic requirements, but considerably below the toxic level for that organ. Toxic effects of oxygen will occur in any cell exposed for long periods to supranormal amounts of oxygen. After 24 hours, an atmosphere of 95 % oxygen damages lipoproteins in the lysosomal membranes of cells in tissue culture, possibly due to inactivation of sulfhydryl groups in enzymes of Kreb's cycle (4). Cellular edema and autolysis result.

The addition of adenine, cysteine and reduced glutathione to culture media has been important in offsetting oxygen toxicity (4).

v. Oxygenators.

An important technical advance in organ preservation has been the oxygenator (7,34,35). These devices permit ready manipulation of perfusate PO_2 so tissue oxygen demands can be met over a wide range of situations.

One problem associated with the use of oxygenators is the generation of emboli by the apparatus (48). Bubbles occur at air-fluid interfaces, and are a particular problem when disc or vertical screen oxygenators are used (34). The membrane oxygenator is more popular because it largely eliminates this problem. A limitation of the membrane apparatus is the relatively large surface area of foreign material in contact with the perfusate, which tends to denature the plasma proteins. Silastic membranes are relatively non-reactive with plasma constituents, and help to diminish the occurrence of emboli (45).

vi. Nutrient Supply.

Little information is available concerning the requirements of hypothermic organs. The kidney has been studied most completely. Acetate, pyruvate, succinate, and glucose are necessary additions for optimal oxygen consumption (7, 49). Insulin is much less active at low temperatures, and may not assist glucose utilization in hypothermic preservations (7). Fatty acids and amino acids are important in renal oxidative glycolysis at hypothermic temperatures. Adenosine in renal preservation, and B vitamins in perfused hearts are thought to increase survival (7,43).

Tissue culture studies reveal that rat intestinal mucosal cells have a high rate of both aerobic and anaerobic glycolysis (50,51). Oxygen consumption is increased by the addition of succinate, pyruvate, citrate, fumarate, or lactate to the tissue

culture solution. In addition to glucose, intestine also metabolizes palmitate, glutamine, acetate and casein hydrolysate (52,53). However, it has not been shown that these substances would support the metabolism of a hypothermic perfused intestine.

While tissue culture studies give some information regarding the hypothermic requirements of organs, in practice, only the addition of glucose and insulin seems of use (28).

vii. Electrolyte Composition of Perfusate.

Physiological electrolyte solutions such as Ringer's, Locke's and Tyrode's are inadequate for perfusion-preservation. Since the concentration of electrolytes in these solutions is identical to extracellular fluid, their failure as perfusates may be due to other factors (54,55,56).

Fluids of varying composition have been tried, and one of the most successful has a composition similar to intracellular fluid. If the perfusate is of this composition, and isotonic with the intracellular fluid, no flux of electrolytes and fluid occurs across cell membranes which may be malfunctioning due to poor preservation or hypothermia. Thus the cellular edema commonly seen may be prevented (55,56,57).

Calcium and magnesium act to maintain the cell membrane by regulating membrane transport of potassium (58,59,60). Citrated plasma is low in calcium and magnesium, and has been reported in liver perfusions to cause cell membrane damage with loss of intra-

cellular calcium magnesium and potassium (61). When the perfusion fluid is provided with intracellular concentrations of these ions, the preservation is better (57).

Calcium and magnesium also have a role in cellular adhesion. A solution deficient in calcium and magnesium will result in the detachment of endothelial cells from each other and from the basement membrane, thus altering capillary permeability (62). Experimentally, it has been observed in lung perfusions, that the use of a hypocalcemic and hypo-magnesemic perfusate results in increased edema (64). Because citrated blood is the source of plasma for perfusates, and is hypocalcemic, magnesium is often added to compensate for the lack of calcium.

vii. Removal of Metabolites and Particles.

Control of metabolites is important in organ perfusion because artificial perfusion systems usually lack the ability to detoxify or excrete waste products. Kidneys preserved with recirculated perfusate are claimed to be damaged from accumulating metabolites and particulate debris in the perfusion circuits (65,66).

Carbon dioxide and lactic acid are probably the most damaging end products to be considered. Their accumulation due to poor tissue perfusion or poor oxygenation can result in acidosis and cellular damage (4).

Vasoactive substances, if allowed to accumulate in the perfusate, increase vascular resistance and make constant flow rates

difficult to maintain. They may be present in large amounts in plasma perfusate collected from a hypovolemic animal because of the inclusion of released catecholamines (4,7).

It is difficult to prevent the occurrence of particles in circulating perfusates, therefore filtering devices are necessary to protect the preserved organ from embolic damage (7).

Trickle perfusion has been suggested to avoid accumulation of metabolites. In one study, a perfusate of hypothermic Collin's solution was not recirculated so toxic metabolites and cellular debris could be eliminated as a source of organ damage (67). Perfusate dialysis has also been suggested as a means of waste removal (47).

ix. Delivery of Perfusate.

a. Autoregulation.

The first principle of isolated organ perfusion is maintenance of an adequate flow rate (7). When normothermic conditions exist, an organ may autoregulate perfusate flow. Autoregulation occurs when an organ adjusts it's blood flow in accordance with it's needs. In Vivo kidney, brain, skeletal muscle, liver, myocardium, small intestine and colon all have this ability (68). Since autoregulation of flow is almost universal in the In Situ organ, it should be considered in the isolated perfused organ, although there are many factors, including denervation and hypothermia, which may alter autoregulatory mechanisms.

Several theories explain the mechanism of autoregulation

of blood flow: tissue pressure, metabolic control, and myogenic control.

1. Tissue Pressure.

Regulation of flow may occur from hydrostatic pressure transmitted to blood vessels from the extravascular compartment. In the intestine, this can be from smooth muscle compression during peristalsis, or from interstitial fluid pressure. Intestinal muscular contraction can markedly reduce flow to the underlying mucosa (69). The effect of tissue hydrostatic pressure on intestinal blood flow is debatable. Hydrostatic tissue pressure transmitted to low pressure capillary and venous vessels may cause partial closure (70). It is claimed that an organ must be enclosed in a rigid capsule for such a mechanism to be effective, and that intestinal capillary flow is not demonstrably affected by tissue pressure (71,72). However, distension of the intestine by intralumenal fluid can reduce vascular flow by compressing blood vessels within the intestinal wall, and obstructing capillary perfusion.

2. Metabolite Control.

When blood flow to a tissue is decreased, acidosis occurs as metabolic by-products accumulate. Accumulating acids, acting directly, or indirectly, may reduce the tone of vascular smooth muscle, thereby decreasing resistance and increasing blood flow (68,73).

3. Myogenic Control.

Vascular smooth muscle responds actively to stretch. When intravascular pressure increases, the vascular smooth muscle may momentarily stretch, but then actively constricts. This is probably the main method of regulating intestinal blood flow (68). Experimentally, resistance to blood flow has been found to decrease in the intestine as the arterial pressure is reduced from 100 mm to 30 mm Hg. A reduction of 25% in resistance in this pressure range is typical. When intestinal venous blood pressure is increased to 25 mm Hg. the vascular resistance increases up to 40%, resulting in reduced flow. These responses to arterial and venous pressure change aid in maintaining a constant flow in the intestine (68).

b. Distribution of Intestinal Flow.

In Vivo canine intestinal flow is reported to be between 0.2 and 2.0 cc/gm/min. (49, 68). Blood flow is not uniformly distributed throughout the intestine. Intestinal flow in the dog has been measured by deuterium oxide, and microsphere techniques. It was observed that two thirds of the blood supply goes to the mucosa - submucosal region, and one third to the

muscle (74). The flow of blood in the intestinal mucosal capillary bed shows periodicity caused by changes in tone of the precapillary sphincter. The precapillary sphincter is very sensitive to changes in intravascular pressure. Intestinal arterioles, on the other hand, are sensitive to changes in metabolite levels, and augment the precapillary sphincter control of flow in the capillary bed (68).

At low perfusion pressures, intermittant cessation of microvascular flow occurs. When the intravascular pressure falls below the critical perfusion pressure, the vessel walls become very unstable, and collapse, emptying their contents into anastomotic vessels. Vascular collapse occurs because the elasticity of the capillary wall, and the interfacial tension between the perfusate and the non-wetting vessel wall overides the perfusion pressure (75).

Thus, autoregulation not only provides optimal pressure-flow relationships, but insures protection of the delicate mucosal microvasculature from the extremes of pressure: vessel closure and capillary disruption. Failure to perfuse a hypothermic organ within physiological limits of pressure is not compensated for by the organ's autoregulative ability, and microvascular damage may be inflicted. Proper pressure and flow in the perfused organ must be substituted for the lack of autoregulation (76).

c. Venous Pressure.

Venous outflow pressures also contribute to organ flow rates. Lung (77), Liver (78), and kidney (7) perfusions have been reported to be more successful when venous outflow pressures were varied from slightly positive to slightly negative. This simulates intra-abdominal pressure changes which are related, in the intact animal, to respiratory activity. Better perfusate flow occurs because a slower axis flow increases pressure enough to open sphincters of lateral branching arterioles (7).

d. Quality of Flow.

Physiological flow rates in the perfused organ are difficult to attain. This is partly due to factors such as hypothermia, inadequate perfusates, and the isolation of the organ with loss of autoregulation. Inadequate perfusion results in a lethal cycle of edema, hypoxia, acidosis, and further edema (7,79). Thus, one must consider factors relating to flow.

Poiseulles' law expresses the relationship of these factors:

$$Q = \frac{\Delta P \pi r^4}{8ml}$$

Q	flow
ΔP	perfusate pressure gradient
r	vessel radius
m	perfusate viscosity
l	vascular length

Poiseulles' law refers to flow in a system of rigid tubes. In organ perfusion, this equation only approximates the description of flow through a vascular bed continuously changing in it's dimensions. The variables in this equation must be considered if adequate flow is to be achieved (4).

Investigations of the quality of flow began with observations on whole body perfusions. In whole body perfusion, pulsatile flow gives better venous return with less external pooling of blood, more physiological distribution of flow in the splanchnic circulation, and a better systemic tolerance to high flow rates and prolonged perfusion (80). There is also a lower mean arterial pressure and less peripheral resistance with pulsatile flow (81, 82).

These observations have been applied to the perfusion of individual organs. Higher flows were obtained when perfusing rabbit ear if pulsatile rather than non-pulsatile flow were used (75). In the kidney, non-pulsatile flow causes arterial straightening and narrowing, resulting in increased vascular resistance and the need for higher perfusion pressures (83). Austen compared pulsatile and non-pulsatile perfusion in normothermic intestine. He noted mesenteric and mucosal hemorrhages progressing after three hours in those organs perfused with non-pulsatile flow. After six hours, the flow in the organs of the non-pulsatile group was reduced, acidosis was progressive, and the segments were

edematous and cyanotic. On the other hand, the intestine perfused with pulsatile flow retained a normal appearance and perfusate flow for the duration of the eighteen hour study (84).

Pulsatile flow is thought to benefit the isolated hypothermic organ. Belzer had the best success in renal preservation when he used pulsatile flow (9). Furthermore, pulsatile flow proved best for liver preservation, giving better gross appearance, perfusate flow, and bile flow than non-pulsatile perfusate flow (85).

The majority of reports concur with the foregoing, however, there is one recent report of a 72 hour non-pulsatile renal perfusion describing excellent results. The authors reported that edema did occur, but suggest that it was due to uncontrolled changes in PO_2, osmolarity, and perfusion pressure, and not related to the non-pulsatile character of flow (86).

e. Resistance.

Increasing resistance seems to be the final common expression of perfusion failure (4). Decreased vascular distensibility caused by hypothermia, extravascular compression due to edema and embolic obstruction all increase vascular resistance (17,23). The radius of blood vessels, reflected to the fourth power in Poiseulle's law is an important factor affecting resistance. Radius in turn is affected by denervation, changes in vascular muscle tone, and tissue pressure (4). Increased resistance secondarily results in reduced tissue perfusion and cellular damage. Through autoregulatory recruitment of more vascular channels,

flow can be increased with small increases in pressure without a change in resistance.

In Vivo vascular resistance in the canine intestine is reported to be about 500 dynes/cm/sec-5 (1).

f. Viscosity.

In hypothermic perfusions, flow tends to be reduced by increased perfusate viscosity. Increased viscosity caused by hypothermia may be corrected by dilution (7), or the addition of dextran, hydroethyl starch, or polyvinylpyrrloedone (87).

x. Perfusates.

a. Whole Blood.

While whole blood is the ideal perfusate in the intact animal, it is not the best in the isolated perfused organ (4,7). Components of the perfusing circuit damage red cells and plasma proteins (5). The trauma to an organ engendered by anesthesia, hypotension, anoxia, and surgical removal may activate the clotting mechanism to generate fibrin (7). Platelets agglutinate when exposed to collagen, epinephrine, or ADP. White cell and red cell sludging is aggravated by activated platelets, fibrin, and hypothermia (14,48,88). Hemoglobin pigment precipitates as hematin in an acid environment forming microemboli (4). Plasma proteins denatured by oxygenator or pump surfaces damages the vascular endothelium of the isolated organ (89). Shed vascular endothelium cell membranes, and other particles from disrupted cells cause emboli (28,89). Lipoprotein precipitates in hypothermic perfusions, and clumps of bacteria occuring in prolonged

perfusions are two other sources of emboli when blood is used as a perfusate.

Embolic complications may be prevented by the inclusion of an appropriate filter in the circuit. A glass wool filter will remove platelets, polymorphonuclear leucocytes, monocytes, and red cell aggregates from heparinized blood (90). Other organs (a second kidney, liver or lung) have been imposed in perfusion circuits to act as filters (91).

Under normothermic conditions, better flow is possible if blood is diluted. Humphries (7) has achieved 48 hour renal preservations, and Pitzele (92) 48 hour heart preservations using dilute blood as a perfusate. Salerno (93) Iijima (94), Austen (84), Hohenleitner (52), and Ruiz (1) all used diluted blood for their short duration, normothermic intestinal perfusions.

Autologous blood gives better results in perfusions than homologous, possibly because of the avoidance of immunological differences (95).

In spite of it's nutritive and oxygen carrying ability, blood presents so many other problems that perfusion-preservation is best done with other media.

b. Plasma.

Plasma is a good perfusate at low temperatures, and currently is the most frequently used perfusate in both experimental and clinical situations. Embolic vascular obstruction is avoided if the plasma is frozen and filtered after rapid thawing

to remove cryoprecipitates (28).

There is some controversy about the addition of other materials to cryoprecipitated plasma. In Vivo, platelets have a role in maintaining the integrity of vascular endothelium. Experimentally in perfused kidneys, platelet-rich plasma has been reported to prevent edema, allowing low perfusion pressures to be used (96).

By regulating the osmolarity or viscosity of cryoprecipitated plasma with dextran, edema and cellular damage can be reduced (6,92).

c. Artificial Media.

Tissue culture media have been devised to propagate cellular replication In Vitro (97,98). They generally give poor results when used as a perfusate in organ preservation. However, some success has been reported when Medium 199, a solution containing various electrolytes in calf serum, was used to perfuse kidneys. 25% survived after four-day perfusion, and 100% survived after one-day perfusion (36). The addition of Eagle's medium to plasma does not increase organ survival (40). Efforts have been made to change the composition of tissue media to create artificial perfusates (99). Better tissue preservation results from the addition of oleic acid, succinate, pyruvate, alpha-ketoglutarate, fumerate, malate, acetoacetate, glucose, cortisone, and vitamin A to standard tissue culture media (7,100).

d. Pharmacological Agents.

One group of pharmacological agents used in organ preser-

vation are those acting as metabolic inhibitors and membrane stabilizers. Of the many theoretically useful agents, only chlorpromazine (101,102,105), and cortico-steroids (23) have practical application in organ preservation. They act in an unknown manner to stabilize cellular, lysosomal, and mitochondrial membranes, and so prevent cellular edema and autolysis.

Another group of pharmacological agents including vaso-active agents, diuretics, buffers, and antibiotics are used to correct secondary problems in organ perfusion. Vaso-active drugs (e.g. procaine and papaverine) may act by causing vasodilation, or they may antagonize such circulating vasoconstrictors as serotonin and catecholamines (4). Arfonad, dibenzyline, dexamethasone, and Periactin have been used to prevent vasoconstriction in perfused organs (4). Anticoagulants such as citrate, heparin and ethyl-enediamine reduce cellular aggregations and prevent vascular obs-truction (4). Diuretics are of importance chiefly in renal perfus-ions (103), however, osmotic agents may be of use in controlling cellular edema in other perfused organs. Buffers such as bicarb-onate and tris (aminomethane) are important in regulating acid metabolic products. Antibiotics may be used to suppress bacterial overgrowth in the perfusing media (4,7).

3. Thrombus Formation.

The prime obstacle to successful vascular microsurgery is thrombosis at the vascular anastomotic site (104). Thrombosis

complicates the re-implantation of preserved organs, and can be initiated by many factors related to the preservation (105). Contact with foreign surfaces of the perfusion circuit components may activate perfusate plasma clotting factors. Damaged cellular elements of the perfusate, especially red cells and platelets, may release ADP which will activate the platelets of the host animal. Non-physiological perfusion will cause destruction of organ parenchymal cells or vascular endothelium, which may result in the release of tissue thromboplastin or platelet activating substances. The reimplanted organ is thus susceptable to intravascular coagulation, both at the vascular anastomotic site, and in it's small vessels where pooling of blood can occur due to sluggish vasomotor control of blood flow. There are two types of thrombosis of interest in the present study, mixed thrombi, and platelet (white) thrombi.

A. Mixed Thrombi.

In areas of sluggish blood flow, contact of blood with a foreign surface activates plasma clotting factors (190). A thrombus is formed when the resulting fibrin enmeshes the cellular elements of the blood. In a re-implanted organ previously damaged by poor perfusion-preservation, there can be tissue destruction with enough stimulation of the host's clotting factors to cause intravascular coagulation.

B. Platelet Agglutination.

White thrombi occur in areas of high fluid shear. If one

observes the events immediately after intravascular trauma, within two minutes, the lumen about the damaged area progressively fills with a yellowish aggregate of platelets, and blood flow is obstructed. Platelet thrombi can occur in both arterial and venous anastomotic sites (107).

A normal platelet is activated by substances present at the site of vascular damage - collagen, ADP, serotonin, epinephrine, and thrombin (108). If it comes into contact with one of these substances, a platelet immediately throws out many pseudopods with sticky bulbous tips (69). In this altered state, the platelets will stick to other platelets, or to a site of endothelial disruption. This process is reversible, and after a time, the platelets may revert to their normal state (110). If the platelet is strongly stimulated, it breaks down into amorphous hyaline material. In a vascular lesion, the platelets are active for about twenty minutes, after which time a basal layer of irreversibly damaged platelets protects the remaining platelets from further stimulation, allowing them to recover and disperse (107).

Platelet aggregation occurs in blood vessels only if the endothelial lining is disrupted, and the basement membrane exposed. If the endothelial cells are damaged without exposure of the basement membrane, no platelet aggregation occurs (III). Material released from the damaged parenchymal cells of certain organs - liver, brain, myocardium and skeletal muscle - induces platelet agglutination without endothelial disruption (109).

Collagen is the only structured intact tissue component shown to cause platelet agglutination (112).

C. Anticoagulation.

Anticoagulants offer a means of preventing coagulation due to tissue damaged by perfusion. Heparin and bishydroxycoumain have no effect on platelet thrombi apart from the prevention of fibrin-stabilization of the platelet plug. Their main effect appears to be prevention of a rapid vascular occlusion and clot propagation (113, 114, 115).

Drugs such as sulphinpyrazone (116), antihistamines (115), dipyridamol (115, 117, 118), adrenocorticoids (114), and acetylsalicylic acid (119), all inhibit platelet agglutination. Dipyridamole used in varying doses from 15 mg/kg/day in rats, to 5 mg/kg/day in man, has been shown to reduce platelet thrombi in response to vascular injury (114, 117). Acetylsalicylic acid in humans inhibits In Vitro collagen-induced platelet agglutination (120). A dose of 100 mg/kg, given 14 to 15 hours prior to study prevents the collagen stimulated agglutination of morphologically normal human platelets (119). Prednisone has been used in doses of 1.5 to 2 mg/kg/day in conjunction with acetylsalicylic acid and dipyridamol to successfully prevent platelet consumption in microangiopathic hemolytic anemia (121). It is postulated that this combination of drugs can be used to prevent vascular coagulation in the perfused organ.

In addition to the above systemically administered anticoagulants, magnesium sulfate is available as a locally applied agent. It has been reported to prevent platelet thrombi if continuously applied in dilute solution at the site of a recently completed vascular anastomosis (107).

4. Vitamin B_{12} Absorption.

Absorption of Vitamin B_{12} has been used to assess changes in absorption from the small bowel in various clinical diseases and laboratory situations (132). In the present experiment, a normal absorption of vitamin B_{12} would verify the ability of the mucosal cells both to secrete binding factors necessary for B_{12} absorption, and to actively absorb the vitamin. For this purpose, the test is probably better than others which use glucose or xylose, because vitamin B_{12} is not passively absorbed, and therefore the appearance of normal levels of B_{12} in blood and urine would indicate that the mucosa has been adequately preserved.

The classical vitamin B_{12} absorption process is not important in the dog (123, 124). Addition of canine gastric juice to a canine intestinal preparation does not enhance the absorption of the vitamin. Rather, it is believed that binders exist in the intestinal mucus of the dog which serve the same purpose as intrinsic factor in other species. If labelled vitamin B_{12} is followed microscopically, it first enters mucus on the surface of intestinal crypts and villi. By two hours it is also seen in the mucus granules of goblet cells in both crypts and villi, and after three hours

appears within the mucosal absorptive cells (124). Thus, it is important to maintain the goblet cell population if vitamin B_{12} absorption is to occur normally after canine intestinal preservation. A mechanism of vitamin B_{12} absorption similar to that of other species also exists in the dog, because administration of intrinsic factor from other species will greatly enhance canine B_{12} absorption (123,125,126). This fact was utilized in the present study of B_{12} absorption.

5. Pathophysiology of Poor Preservation.

A. General.

Rising perfusion pressure, indicating increased vascular resistance, is usually due to embolization or vasospasm. If plasma is used as a perfusate, the emboli are usually derived from denatured lipoproteins; if blood is used, the cellular constituents are a source of emboli (7,28,102). Embolic obstruction reduces capillary flow which in turn results in tissue hypoxia. Cells are then forced to use anaerobic metabolic pathways, the products of which accumulate to create an acid environment (127). The cell membrane loses its selective permeability and cellular edema results. The edema is accentuated by hypothermic inactivation of membrane stabilizing enzymes. There is an increased rate of leakage of lysosomal enzymes across the lysosomal membrane (13,106), although this harmful effect may be partly offset by the hypothermic inactivation of the hydrolytic enzymes so released (127). Difference in metabolic rate and in the metabolic pathways may be due to

resistance to trauma of the mucosa and submucosa of the bowel. This is shown by differences in succeptability to the hypoxic and traumatic events in preservation (128). Therefore, inadequacies in perfusion preservation are first and most profoundly manifest in mucosal damage (128).

B. Observations with Perfusion Preservation.

See Appendices 1 and 2 for perfusion parameters of the following reports.

Rat intestine has been a good model to demonstrate the gross effects of poor perfusion with normothermic blood. Hyperemia, hyperperistalsis, hypersecretion, and mucosal cell necrosis occur (76). Hyperemia begins about 30 minutes after the start of the perfusion. It is associated with a low vascular resistance so that the flow rate must be increased to maintain the desired perfusion pressure. After 15 minutes of perfusion, strong spasmodic contractions appear in all regions of the intestine. During the perfusion, the intestine becomes progressively distended due to the accumulation of fluid in the lumen. Hyperperistalsis appears, and continues until the bowel is severely distended with fluid. (The electrolyte composition of the fluid is similar to that of plasma; the protein content is 2 mg/cc). By one or two hours the bowel is turgid. There is no fluid loss from the serosal surface and no increase in lymphatic flow. Histological examination reveals mucosal epithelial necrosis after five hours of perfusion (76).

Kavin also noted loss of isosmotic perfusate

into the intestinal lumen when he perfused rat bowel with blood diluted with low molecular weight dextran (129). If peristalsis was vigorous, there were associated mucosal hemorrhages and poor bowel viability.

Alican (2) perfused total canine small intestine with homologous serum at 9°C for 24 hours. Gross and microscopic observations at the termination of the perfusion were not reported. However, hyperperistalsis and mucosal slough occured when the bowel was allografted, and the experimental animal experienced the rapid onset of lethal shock.

When Hohenleitner (52) perfused canine intestine with sheep red cells in an electrolyte solution at normothermic temperatures, he noted occasional vigorous peristalsis and marked accumulation of fluid in the lumen.

Ruiz (1) performed pulsatile perfusion of dog intestine with autologous normothermic dilute blood. He noted weight gain of less than 5%, no spontaneous peristalsis, and a normal histological picture after two hours of perfusion. The segments of bowel in his experiment showed gradual loss of enzymes into the perfusate (LDH, SGOT, acid phosphatase and B-glucuronidase) during the perfusion, suggesting cellular damage.

Salerno (93) perfused segments of sigmoid colon for five hours at room temperature with blood diluted with Ringer's lactate. A gradual rise of sodium, potassium, chloride, and a fall in pH occured in the perfusate. High perfusion pressures

(over 140/70 mm Hg) were associated with mesenteric hemorrhages and distension of the bowel with lumenal fluid. In all segments at the end of five hours, edema was noted, especially in the submucosa. An attempt was made to reimplant segments of perfused colon. This was unsuccessful because of subsequent thrombosis at the vascular anastomosis. The difficulty in attaining a functional vascular anastomosis in an apparently viable perfused organ is to be emphasized. Adequate anticoagulation is necessary for successful reimplantations (94).

6. Introduction to the Experimental Problem.

Reported success of perfusion preservation of the small intestine has been limited. Most experimental attempts have employed pulsatile perfusion with normothermic blood (1,76,94,130, 131). There is only one reported experimental perfusion-preservation, this with hypothermic serum (2). Success in most studies were evaluated by gross and histologic observation, reimplantation, and the ability of the bowel to absorb xylose or glucose either during preservation or after reimplantation. The longest (normothermic) perfusion resulting in viable intestine (tested by In-Vitro absorption) was 18 hours in duration (84). The longest perfusion tested by successful reimplantation was only 5 hours in duration (94). The lack of success probably is due to the methods of preservation used. Whole blood is not a good perfusate for organ preservation and the use of cryoprecipitated plasma may give better

results (28). Maintenance of the preserved organ after reimplantation is important, particularly in respect to thrombosis of the graft. A regimen of anticoagulation must be instituted (94). Also, a more precise test of the functional ability of the preserved intestine would be useful to evaluate the quality of preservation.

In the present experiment, perfusion-preservation of small intestinal segments was studied using pulsatile perfusion with hypothermic cryoprecipitated plasma. General principles found useful in renal preservation and from limited experience with intestinal preservation were reported in the literature to these incorporated experiments.

7. Description of Experimental Groups.

Group I. Control series of four dogs in which segments of terminal ileum were excised, flushed of blood with a solution of heparinized saline and immediately reimplanted. No anti-coagulation was used.

Group 2. The nine dogs in this group had segments preserved four hours. The oxygenator, addition of chlorpromazine, and anti-coagulation were not used.

Group 3. The four segments in this group were perfused for eight hours without the use of an oxygenator or chlorpromazine. Anti-coagulation was used.

Group 4. The fifteen segments in this group were perfused

TABLE 2. Experimental Groups.

I. Control-excision and immediate autoimplantation of bowel.

2. Four hour preservation: oxygenator and chlorpromazine not used.

3. Eight hour preservation: oxygenator only.

4. Four hour preservation: oxygenator and chlorpromazine used.

5. Eight hour preservation: oxygenator and chlorpromazine used.

6. 24 hour preservation: oxygenator and chlorpromazine used.

eight hours with the aid of the oxygenator and chlorpromazine. Anti-coagulation was used in all but three instances.

Group 5. The four dogs in this group had segments perfused four hours using the oxygenator in the circuit. Chlorpromazine was added to the perfusate. The anticoagulation regimen (Page 48) was used.

Group 6. The six segments of this group were perfused 24 hours using the oxygenator, chlorpromazine, and anti-coagulation.

8. Methods.

A. Apparatus.

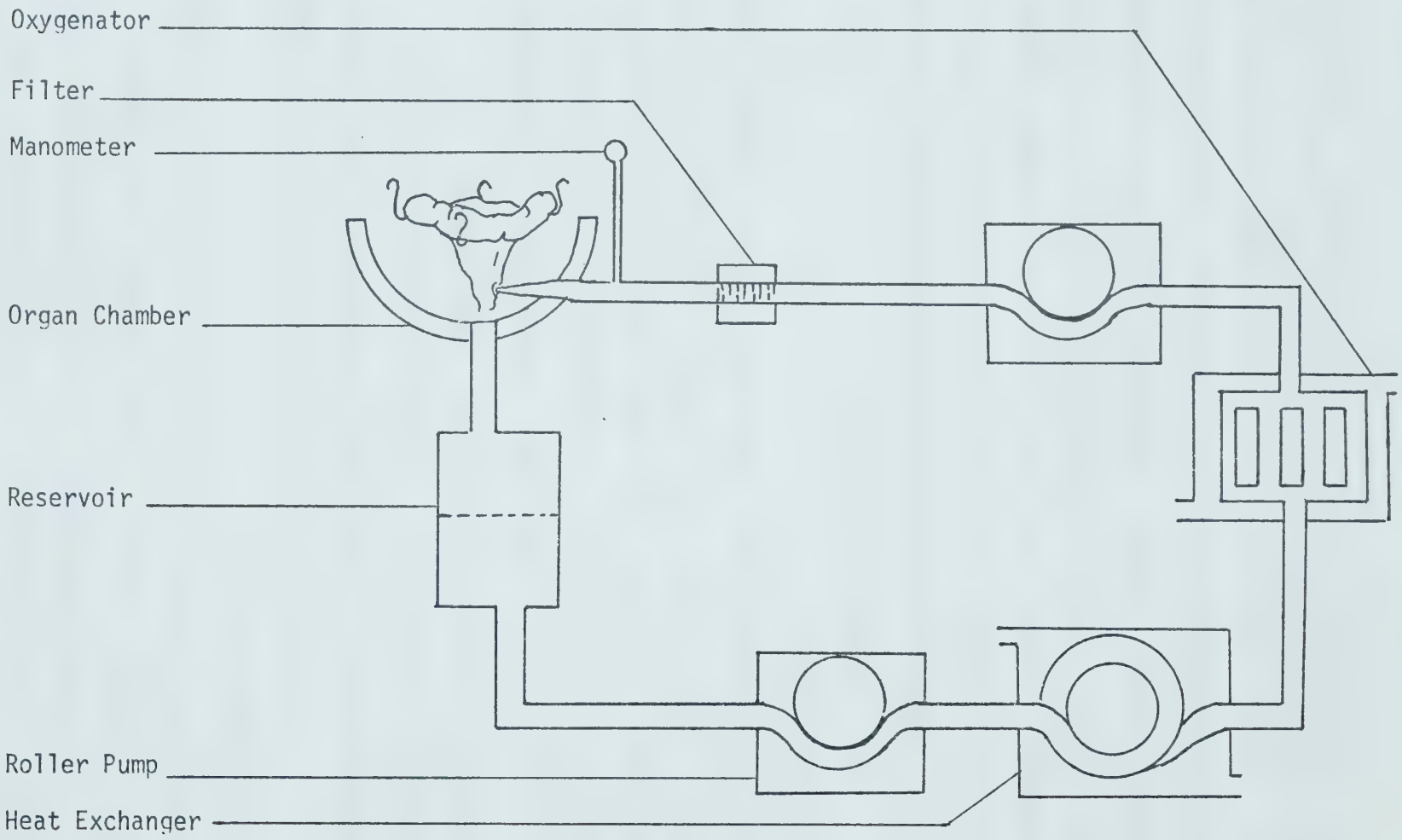
A perfusion system was designed, comprised of: an organ platform, a pump delivering pulsatile flow, a membrane oxygenator, an in-line filter, a heat exchanger, and perfusate reservoirs. (See illustrations 1 and 2). All parts of the circuit in contact with the perfusate were made of either stainless steel or silastic. These materials were used because they are relatively inert (132).

The organ was housed in a 25 by 30 cm double-walled, closed, transparent, acrylic chamber to help maintain a constant temperature and humidity. Initially the intestine was placed on a silastic rack; but later, a silastic tube was passed through the lumen to allow collection of lumenal fluid, and the bowel was suspended from the circumference of the chamber to allow unobstructed venous drainage.

The occlusive roller pumps* were calibrated to provide pulsatile flow of 50 cc/min. Pumping pressures were monitored with an aeroid manometer** attached to the arterial circuit just outside the organ chamber.

* Roller pumps manufactured by Med-Science Electronics, St. Louis, Mo.
** Manometer manufactured by Thistle Co., St. Louis, Missouri.

Illustration 1. Perfusion Apparatus



The heat exchanger was constructed by placing a jacket about a coiled segment of the perfusion line. A refrigerated solution of methyl alcohol was circulated through the jacket.

The entire perfusion circuit was composed of about ten feet of 3/16 inch diameter silastic tubing*. An intravenous cannula** with flanged tip was used to connect the inflow tube to the intestinal artery.

The membrane oxygenator*** consisted of a silicon rubber membrane capable of fulfilling its functions at a low flow and hypothermic temperatures. 100% oxygen was supplied to the oxygenator at 600 cc to 800 cc/min. (45,133,134).

The initial plasma volume necessary to prime the system was 300 cc. The volume in the oxygenator was 75 cc, that in the tubing was 60 cc, about 20 cc were in the organ at any one time, and the remainder was in the reservoir. Because of loss of fluid into the intestinal lumen, there was a complete renewal of perfusate about every 6 hours.

B. Perfusate Preparation.

Homologous pooled canine plasma was prepared in the manner described by Belzer (28). Five Hundred cc alloquots of blood were collected in bottles containing 75 cc of acid citrate dextrose. The blood was centrifuged at 10,000 RPM for 30 minutes and the plasma stored in teflon bottles. 200,000 IU of penicillin G and 0.25 gm of dihydrostreptomycin**** were added to each litre of plasma. The

* Silastic tubing manufactured by Dow Chemical Co. Canada Ltd.
** Intravenous Cannula manufactured by Argyl Co., St. Louis, Missouri.
*** Membrane oxygenator #5M0321 supplied by Travenol Lab. Inc.
**** Penicillin and dihydrostreptomycin, Cambiotic, supplied by Pfizer Co., Ltd., Montreal, P.Q.

plasma was then frozen. Before use, it was rapidly thawed (10 minutes), and the white precipitate filtered off. Initially it was filtered with a 0.22 micron millipore filter*, however, a fine nylon mesh was substituted and found to be satisfactory. Freezing, thawing and filtering were repeated two or three times. This effectively cleared all particulate material.

The concentration of sodium and potassium, and the plasma osmolarity were adjusted respectively to 140 to 150 mEq/l; 3.5 to 5.5 mEq/l; and 290 to 330 mOsm/l. Just prior to use, 4 mEq of magnesium sulfate, 100 mg of hydrocortisone and 80 units of insulin** were added to each litre of plasma. The pH was adjusted to 7.35 to 7.45 prior to use and maintained in this range during the perfusion by the addition of sodium bicarbonate (44 mEq/l). In later experiments, 12.5 mg of chlorpromazine*** were added to each litre of perfusate.

Bacterial growth in the perfusate was periodically checked at the termination of experiments using slide preparations stained with Gram's stain.

* Millipore filter manufactured by Millipore Corp., Bedford, Mass.
** Hydrocortisone and Toronto Insulin Supplied by Connaught Medical Research Laboratories, University of Toronto.
*** Chlorpromazine, Aceprozine, supplied by J. Webster Laboratories Ltd., Toronto.

C. Calculations.

1. The oxygen consumption was calculated from the PO_2 of arterial and venous samples of perfusate using the following formula (15, 133).

O_2 consumption (cc/gm of intestine/min)=

$$\frac{\text{flow (cc/min)} \times Pa_{O_2} - Pv_{O_2}\text{ (mmHg)} \times \text{solubility coefficient}}{\text{atm press (mmHg)} \times \text{bowel weight (gm)}}$$

2. Vascular resistance (R) was calculated according to the following formula: R = (Pi - Po/venous blood flow) x 1333, where Pi is the mean arterial inflow pressure which was constant, Po is the venous pressure which was zero since there was free drainage, and 1333 is the conversion factor for mm Hg to dyne/sec/cm-5 (I).

3. For the calculation of vitamin B_{12} absorption, the amount of radioactivity in the collected urine was expressed as a percentage of the administered dose (135).

D. Preparation of Perfusing Apparatus.

When not in use, the perfusion circuit was filled with a 1% formalin solution. Prior to use it was rinsed with ten litres of distilled water. The membrane oxygenator was stored containing a solution of 5% dextrose in saline to which was added 200,000 units of penicillin G and 0.25 gm of dihydrostreptomycin per litre. This was rinsed out with 1 litre of distilled water before use.

The perfusion circuit was assembled and rinsed with about 100 cc of perfusate which was then discarded. The apparatus was primed with about 300 cc of perfusate. The perfusate was continuously circulated before placement of the organ in the circuit to allow stabilization of temperature, PO_2 and pH.

E. Surgical Preparation.

Dogs weighing 15 to 20 kg were anesthetized with 30 mg/kg of intravenous sodium pentobarbital*, and 12.5 mg of intramuscular chlorpromazine. During the operative procedure, 1000 to 1500 cc of 5% dextrose in water was given intravenously.

Segments of terminal ileum were prepared in the following way. Through a long midline abdominal incision, the artery and vein supplying a 60 to 80 cm length of terminal ileum were isolated and dissected free from the surrounding tissues. The proximal vascular pedicle was lightly occluded with atraumatic bulldog clamps or ligatured if the organ was to be perfused for 24 hours.

* Sodium Pentobarbitol, Diabutal, supplied by Diamond Laboratories, Desmoines, Iowa.

The artery and vein were then cut and the segment of bowel excised. A solution of 5% dextrose in saline containing 6000 units of heparin* per litre, and buffered with $NaHCO_3$ to pH 7.4 was infused through the artery to flush the blood from the segment. A 20 cc syringe was used for this purpose, the infusion being done slowly, but with no attempt to control injection pressure. The segment was weighed. The lumen of the bowel was flushed with saline to clear it of intestinal content. A perforated silastic tube was threaded through the lumen. Metal hooks used to suspend the bowel were passed around the bowel and through avascular sites in the mesentary. The ends of the bowel were tied about the supporting tube to seal the lumen. Finally the divided artery and vein were cannulated with flanged catheters. The intestine was placed in the perfusion chamber. (Illustration 3).

After perfusion, the bowel was drained of remaining lumenal fluid, and reweighed. The crushed ends of vessel and bowel were excised. Vessel re-anastomosis usually took from 30 to 50 minutes, and was done with continuous 7-0 silk sutures (104).

The previously divided mesenteric lymph nodes were re-joined in order to prevent traction on the vascular anastomosis. The ends of the reimplanted bowel were brought through separate incisions in the abdominal wall to form the stomata of Thiry-Vella fistulae. The divided ends of the host bowel were joined by end-to end entero-enterostomy, and the abdomen closed. If the per-

* Sodium Heparin Supplied by Connaught Med. Research Lab., Toronto.

fusion was of 24 hours duration, the host intestine was joined, the abdomen closed, and the animal allowed to run free until the next day when it was re-anesthetized, and the perfused segment of bowel reimplanted.

F. Anticoagulation.

To combat vascular thrombosis after reimplantation, magnesium sulfate, heparin, acetylsalicylic acid, dipyridamol and prednisone were used. Isotonic magnesium sulfate solution was applied to the anastomotic line at one drop per second for 20 minutes with intermittant pinching of the vessel in an attempt to disperse platelet agglutinations at the anastomotic sites.

A combination of acetylsalicylic acid, 150 mg/kg; dipyridamole, 4 mg/kg; and prednisone 1.5 mg/kg was given so that the animal received it 24 and ten hours prior to the vascular anastomosis. Postoperatively, heparin, 100 units/kg was given every four hours for the first day.

G. Procedure for Vitamin B_{12} Study.

This test followed a format previously developed in this laboratory (136). Dogs tested were fasted overnight. A Foley urinary catheter was inserted into each stoma, and the bulbs inflated to occlude the lumena. A solution consisting of 1 microcurie of vitamin B_{12} labelled with Co^{57}, 25 mg of human intrinsic factor, and 500 mg of calcium gluconate was infused into the proximal bowel stoma in 10 cc of physiologic

saline. Two hours later, an intramuscular "flushing" dose of 1000 mcg of vitamin B_{12} was given. The test solution was left in place for 24 hours. During this period, the dog remained in a metabolic cage and total urine output collected. Five 2 cc alloquots of the urine were counted in order to estimate the amount of radio-activity excreted. The amount of radioactivity in the urine was expressed as a percentage of the administered dose. Tests were begun one week after the reimplantation of the preserved bowel. (see graphs 3 and 4).

H. Format of Observations.

1. Parameters of Perfusion.

During the perfusion, perfusate temperature, flow rate, pressure, PO_2, pH, and volume of lumenal fluid were measured. Temperature was measured with thermometers placed in the venous outflow stream and on the antimesenteric border of the intestine. Flow rate was controlled by precalibrating the roller pumps. Outflow was periodically measured by timed venous collections. Pressure was measured with an aeroid manometer open to the arterial line. Periodic collections of perfusate were taken through arterial and venous cannulae and analyzed for pH, PO_2, and PCO_2**. Lumenal fluid was continuously drained and hourly volumes recorded. The concentration of sodium and potassium of pooled samples of this fluid was determined. Weight gain was found by subtracting pre-perfusion

* counting was done on a Mark I (model 6860) liquid scintillator computer, Nuclear-Chicago Corp.

**pH, PO_2, were analyzed using a Radiometer electrode, Canadian Laboratory Supplies Ltd., Toronto.

bowel weight from the weight post perfusion.

2. Gross Observations.

Immediately after blood flow was re-established in an autografted segment, several gross observations were made. The return of color, temperature, and peristalsis were noted and timed. The emission of exudate from the stoma was described, as was the occurance and extent of perivascular edema and hemorrhages.

3. Histologic Observations.

Biopsies of the intestinal segments were taken before perfusion, immediately after perfusion, 30 to 45 minutes after vascular anastomosis, and thereafter through the stomata at intervals of several days. The biopsies were fixed in 10% buffered formalin.

Tissue sections were stained with hematoxylin and eosin. The following morphological characteristics were evaluated; general architecture, height of villi and appearance of epithelial cells. (See table 3 for a list of observed histologic lesions used to compare biopsies).

4. Long Term Observations.

Five dogs with intestinal segments perfused four hours (three of group two and two of group four), and two dogs with segments perfused 8 hours (Group five), were kept for two months postoperatively to observe the effects of perfusion on the intestine. Three weeks after perfusion, animals of group

TABLE 3. Histological Lesions Incurred by Perfusion.

Mucosa	Edema
	Cellular Lysis
	Loss of Mucosal Epithelium
	Disruption of Villus Architecture
	Hemorrhage, or vascular Congestion
Submucosa	Edema
	Vascular Congestion or Thrombi
Muscle	Perivascular Edema
	Cellular Necrosis
Blood Vessels	Perivascular Edema
	Perivascular Hemorrhage
	Congestion or Thrombi

two had their Thiry Vella fistulae re-established in continuity with their alimentary tracts. This was done to establish whether or not these segments would function normally relative to intestinal motility and absorption.

9. Experimental Results.

A. Reimplantation Survival.

See Table 4 for summary of preservation attempts.

B. Perfusion Modalities.

1. Temperature.

The organ took about ten minutes to cool and subsequently maintained a constant temperature of 10°C. There was no temperature gradient from the central (venous outlet) to the peripheral parts of the bowel (antimesenteric border).

2. Flow Rate.

The flow rate was predetermined by the speed of the roller pump, and remained constant at 50cc/min throughout the perfusion. This provided flow rates of 0.2 to 0.8 cc/gm/min. Timed venous collections indicated the venous outflow volume to equal the precalibrated pump flow rate. Organ edema had no apparent effect on the rate of venous outflow.

3. Pressure

The arterial pressures were maintained at 40 to 60 mm Hg systolic and 20 to 40 mm Hg diastolic. The mean arterial pressure was lower in shorter segments of perfused bowel than in longer ones.

TABLE 4.

SURVIVAL OF PERFUSED BOWEL.

Group No.	Attempts	survival 10 days or more	percent survival
1	4	3	75.0
2	9	4	44.4
3	4	4	100
4	4	0	0
5	15	6	40.0
6	6	0	0

4. Vascular Resistance.

See Table 5. The calculated vascular resistance in the perfused segments was about 350% greater than that reported for normothermic In Situ intestine. It gradually diminished during the perfusion. By the end of a 24 hour perfusion, the vascular resistance was about 67% of that at four hours.

5. Perfusate Oxygenation.

Groups of organs were perfused both with and without an oxygenator, and the oxygen consumption observed in each instance. The effect of chlorpromazine on oxygen consumption was also observed. The mean perfusate arterial and venous PO_2's were respectively 114 mm Hg and 62 mm Hg when no oxygenator was used. The addition of an oxygenator increased the mean arterial and venous PO_2's respectively to 630 mm Hg and 404 mm Hg, and was associated with an increase in the mean oxygen consumption (373%). Addition of chlorpromazine to the perfusate of circuits in the oxygen consumption (the mean increase being only 247%). See Table 6 and Graph 2.

6. Weight Gain.

At the end of perfusion, all segments had gained weight. The gain of 60.0% in the segments perfused for 24 hours was significantly greater than that exhibited by segments perfused for shorter periods. See Table 5.

TABLE 5.

Percent Weight Gain (gm)			Vascular Resistance (dyne/sec/cm-5)		
HRS. PRESERVED	MEAN	SD	MEAN	SD	NO. OF TESTS
4	38.6	29.2	1862	122	13
8	27.6	11.8	1555	573	19
24	60.0	24.8	1264	378	6

TABLE 6.

Perfusate PO_2

	ARTERIAL (mm Hg) MEAN	SD	VENOUS (mm Hg) MEAN	SD	NO. OF TESTS
no oxygenator	162	20.8	114	34.2	13
Oxygenator	630	180	404	85.1	25

7. Lumenal Fluid.

These observations are limited to those segments perfused using the oxygenator, and chlorpromazine. The lumenal fluid did not drain at a constant rate. In the first four hours it averaged 26 cc per hour. In the following six hours, the rate almost doubled to 57 cc per hour. It continued to drain at this rate for the next six hours, then increased markedly to 135 cc per hour over the final eight hours of the 24 hour perfusions. The concentration of sodium and potassium in the lumenal fluid did not vary significantly from that of the perfusate. See Graph I.

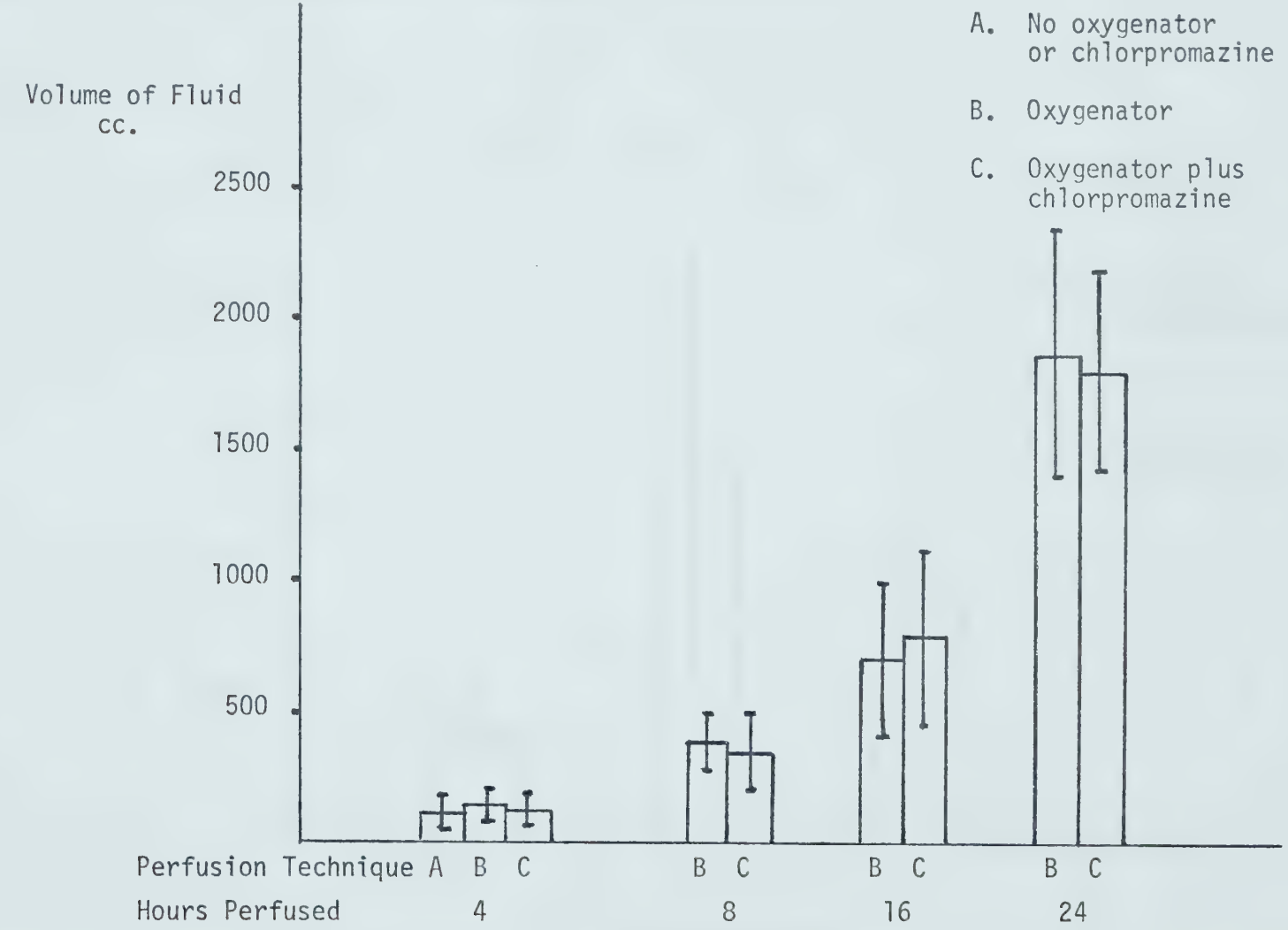
C. Surgical Preparation.

Removal of the segment usually took thirty minutes. The period of ischemia from removal of the segment to institution of the perfusion-preservation was about three minutes. The average diameter of the segment's artery was 2 to 3 mm and that of the vein was 3 to 4 mm. When anticoagulation was begun preoperatively, special care had to be taken with dissection to avoid hemorrhage.

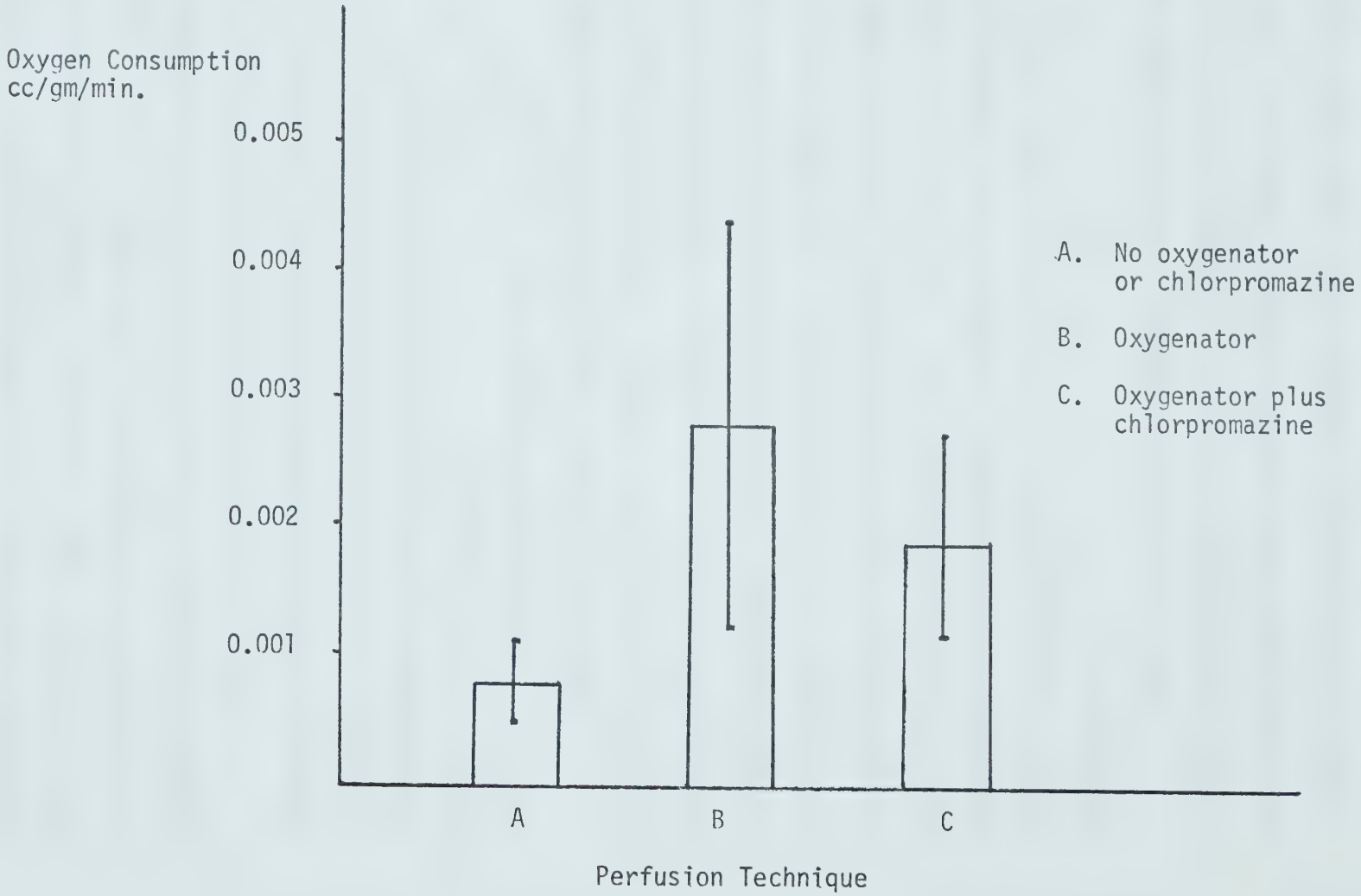
D. Perfusate.

The cryoprecipitated plasma perfusate seemed to function well with the hypothermic preparation of ileum. Easily regulated PO_2's were possible. At the termination of the perfusions, there was no evidence of precipitate in the filter.

Graph 1. Volume of Fluid in Intestinal Lumen



Graph 2. Oxygen Consumption of Intestine During Perfusion



During the perfusion, the perfusate sodium and potassium concentrations did not vary significantly from the pre-perfusion concentrations. These observations, however, are probably not useful because continuous loss of perfusate into the intestinal lumen made necessary frequent additions of fresh perfusate.

No evidence of bacterial growth in the perfusate was detected either by perfusate discoloration, or by Gram's staining of perfusate samples at the termination of perfusions. It is assumed that bacterial growth in the perfusate was not a problem in this experiment.

E. Observations of Bowel During Perfusion.

After the bowel was placed in the perfusion chamber, several observations were recorded. The color was grey-white except for occasional bright red areas in the mesentary caused by small hemorrhages incurred during excision. The bowel was translucent so that any dark material in the lumen could be seen. Pulsations were apparent in the mesenteric vessels and those extending onto the intestinal wall. The intestine was completely immobile and without response to mechanical and chemical stimulation (pinching, and 0.5 cc 10 -4 M acetylcholine intramuscularly).

After two hours of perfusion, progressive edema became evident in the perivascular tissue, becoming maximal by the end of four hours, and remaining constant thereafter. The tissues were not markedly distended, and in no instance did the venous outflow appear to be obstructed. Accumulation of intra-

lumenal fluid has been discussed.

After six to eight hours, white mucus and sloughed villus tips appeared in the collections of lumenal fluid. Concommitant with the increase in intralumenal fluid was the appearance of a clear fluid transudate of the serosal surface. The rate of loss of serosal fluid was less than 5 cc per hour.

F. Reimplantation of Bowel.

When the vascular clamps were removed after the vessel anastomoses, the bowel immediately assumed a normal color. It's temperature equaled that of the host animal within one minute. Peristalsis occured spontaneously in all cases. In segments perfused four hours, it occured between 30 and 60 seconds after blood flow was restored, and was of normal tonicity and rate. Peristalsis continued in this fashion during the two to four hour observation period after the anastomoses were made. In the segments perfused for eight hours, the onset of peristalsis occured about three minutes after completion of the anastomosis. The character of peristalsis was never normal during the initial two to four hours. There was a tendency to hyperperistalsis with vigorous rushes or sustained segmental contractions. In the segments perfused for 24 hours, spontaneous peristalsis occured in three minutes and tended to be vigorous. After two to three hours these segments became hypoperistaltic or completely flaccid.

Within five minutes fluid appeared from the stomata.

Usually it was clear, straw colored fluid containing mucus and sloughed mucosa cells. The amount of mucosa seemed to depend on the vigour of peristalsis. In the first hour, about 100 cc of fluid appeared; thereafter the rate of efflux was about 15 to 20 cc/hour. If the mucosa was poorly preserved and sloughed, the exudate contained gross blood.

Intestinal segments perfused for four and eight hours had perivascular edema which increased over that accumulating during the perfusion. It did not appear to cause vascular obstruction, and usually had completely subsided by three hours. Intestine perfused for 24 hours had marked edema so that the perivascular tissue was turgid and firm to the touch. However, no vascular obstruction was apparent and this edema also subsided by three hours.

G. Failed Segments.

Group I.

The one survival failure in this group was attributed to thrombosis at the site of venous anastomosis. It was felt that the mesenteric lymph nodes along the vascular pedicle of the preserved bowel compressed the vein and initiated thrombosis.

Group 2.

Failure of survival of four segments in this group was probably caused by white thrombi occurring at the arterial anastomotic site. In one dog, the joined mesenteric lymph nodes

supporting the vascular pedicle spontaneously separated. Narrowing and damage at the site of anastomosis due to traction probably contributed to thrombosis.

Group 3.

Failure of survival of all segments in this group was felt to be due to poor tissue preservation. All blood vessels of each segment were occluded with mixed thrombi which extended from the vascular anastomotic site to the bowel wall.

Group 4.

The success of this group was attributed to the combination of the good tissue preservation and the anticoagulation regimen.

Group 5.

A variety of problems contributed to the failure of these segments. In two instances, it was impossible to complete the arterial vascular anastomosis due to small vessel size. Three failures were attributed to uncontrolled hemorrhage from the arterial anastomotic site, probably secondary to anticoagulation. The remainder failed due to white thrombi occurring at the arterial anastomotic site, in spite of the anticoagulation regimen.

Group 6.

Two subjects of this group succumbed to uncontrolled hemorrhage from the arterial anastomotic site. One failure was attributed to vascular thrombosis produced by twisting of the perfused intestine about the axis of its vascular pedicle. The

remainder of the segments showed widespread intravascular thrombosis of the perfused segment, similar to that occurring in group 3.

H. Gross Observations.

In the control group, and those surviving in groups one, two, four and five (four and eight hour perfusions), the stomata of the Thiry-Vella vistulae initially were dark pink or purple. Normal pink color returned by the second or third post operative day. Thereafter, the stomata remained a normal color, continued to secrete clear fluid, and exhibited peristalsis throughout the three week (control) or two month observation periods.

The stomata of those segments unsuccessfully preserved became black and dry within the first 24 hours postoperative.

Two months post-perfusion, segments of bowel were examined at laparotomy. They were bound with adhesions, and the mesentary was thickened and contracted. The serosal surfaces were firmly united to each other and bled when attempts were made to separate them. In those dogs which had the perfused segment re-established in continuity with the alimentary tract, intestinal dilatation was evident proximal to the autografted segment, indicating partial intestinal obstruction. This appeared to be the result of adhesions kinking and obstructing the bowel, rather than stenosis at the intestinal anastomotic site.

I. Vascular Thrombosis.

There were several distinct patterns of thrombosis within the vessels of the perfused intestines. Mixed thrombi were noted

in both arteries and veins, either in association with compression or kinking of the vascular pedicle, or with histologically obvious poor tissue preservation. They occured within 48 hours of re-implantation.

Those thrombi due to compression were usually confined to the larger arteries and veins of the graft pedicle, and extended for six to eight cm proximal and distal to the site of vascular obstruction. In such segments, thrombi usually did not reach to the distal vascular arcades supplying the bowel. These thrombi usually occured in the first 24 hours post reimplantation and were only adherent to the vessel lumen at the site of vascular obstruction.

Segments poorly preserved had mixed thrombi filling all blood vessels, and extending to the first major vessel junction proximal to the vascular anastomosis. These thrombi were adherent to the vessel wall in many areas and were difficult to express from the vessel lumen. They occured within 24 hours of reimplantation.

White thrombi were found mainly at the arterial anastomotic site. They were localized, adherent to the suture line, and extended one or two cm proximal and distal to the suture line. Usually they were associated with a short length of mixed thrombus beginning at the distal end of the white thrombus and extending several cm along the main artery.

J. Histological Observations.

Group I. Control.

In this group of three dogs, biopsies were taken from the stomata of reimplanted intestine twenty minutes post implantation, and at intervals of several days for three weeks. For the first two to three days after operation, mild edema and vascular congestion occurred in the villi. There was no mucosal epithelial slough. At the end of the three week observation period, biopsies appeared to be normal with the exception of occasional slightly shortened villi.

Group 2. Four Hour Perfusion: Oxygenator and Chlorpromazine not used.

Sequential biopsies were obtained on nine segments. The post perfusion, pre-implantation biopsies showed the intestinal mucosa to be the tissue most damaged. Moderate edema of villi with elevation or sloughing of the mucosal epithelium was common. Disruption and loss of up to 25% of distal villus tip length occurred frequently. The mucosal cells of the intestinal crypts appeared to be well preserved. The goblet cells were more pale staining than normal with less evidence of granular markings. There was moderate edema of tissue immediately beneath the muscularis mucosa, and perivascular edema within the muscularis.

Biopsies obtained twenty minutes after reimplantation showed mucosal blood vessels congested with red cells. Blood vessels within the submucosa and muscularis also contained

occasional clumps of polymorphonuclear leucocytes adhering to the inner walls. Where villus tip loss had occured, blood was observed in the intestinal lumen. However, when the histologic appearance was compared with that of the initial biopsies, no further damage could be seen.

Intestinal biopsies taken one to three days post-operatively showed that only about 25% of the villus length remained. The only intact mucosal cells were those in the crypts. They appeared to be low, columnar and darker staining than normal. Over a period of seven to ten days, the length of the villi increased to about 75% of normal. At this time they were covered with normal appearing epithelium. Crypt cells also regained a normal appearance.

Biopsies taken toward the end of the two month observation period showed a normal appearing histologic picture with normal villus length and a normal mucosal epithelium.

Group 3. Eight Hour Perfusion: Oxygenator.

After perfusion, the four segments in this group showed poor mucosal preservation. The mucosal epithelium was almost completely sloughed, leaving only the basal cells of the crypts. They were dark staining, shrunken and had pyknotic nuclei. Villus edema was marked, with loss of at least 50% of villus length. There was also moderate edema of the submucosa and perivascular spaces in the muscularis.

On revascularization, there was vascular congestion and hemorrhage from the torn ends of sloughed villi.

By 24 hours, post-perfusion, all segments were necrotic because of thrombosis at the arterial anastomotic site.

Group 4. Four Hour Perfusions: Oxygenator and Chlorpromazine Used.

Four segments were studied in this group. The post-perfusion biopsies showed better preservation than was observed in group two. There was only slight edema of the villi and most of the mucosal epithelium was intact. There were occasional areas where slough of mucosal epithelium at the villus tips had occurred. There was only slight edema of the submucosa and perivascular spaces of the muscularis.

Biopsies were taken immediately after reimplantation and showed no change in the above picture.

Biopsies taken one to three days post operatively showed a loss of about 50% of distal villus length. The remaining mucosal cells were low columnar and slightly darker staining than normal. After ten days the length of the villi was normal or slightly shortened, and the mucosal epithelium appeared normal.

At the end of two months, biopsies showed normal villus length and mucosal epithelium.

Group 5. Eight Hour Preservation: Oxygenator and Chlorpromazine Used.

Histologic study of the fifteen segments in this group showed that they were better preserved than those of group three.

Immediately post perfusion, there was slough of about 25% of the mucosal epithelium from the villus tips. In all instances, the cells of the crypts were well preserved. There was slight villus edema and only an occasional villus tip lost. There was also slight edema of the submucosa and the perivascular spaces in the muscularis.

After reimplantation, there was hemorrhage from the damaged villi, but no apparent deterioration of the histological picture.

Biopsies taken during the first to third postoperative days showed short villi with only the basal mucosal epithelium of the crypts remaining. They were low cuboidal, shrunken, and had dark staining cytoplasm.

By ten days, the villi were about 75% of their normal length, and covered with normal appearing mucosal epithelium.

Biopsies taken at 2 months showed normal villus length and normal mucosal epithelium.

Group 6. 24 Hour Preservation: Oxygenator and Chlorpromazine Used.

The six segments perfused were very poorly preserved. In the post-perfusion biopsies, there was slough of almost all the mucosal epithelium. The mucosal cells of the crypts were shrunken, dark-staining, and had pyknotic nuclei. There was loss of about 75% of the distal villus tips and the remaining villus bases were markedly edematous. There was marked edema of the

submucosa and muscularis. The myocytes of the muscularis were light staining with loss of striation.

Revascularization was associated with hemorrhage from the sloughed mucosal tips, and prominent perivascular hemorrhages in the submucosa and muscularis.

Stomal biopsies taken on the first and second post-perfusion days showed complete slough of mucosal epithelium and villi, and marked hemorrhages into the now necrotic muscularis.

Summary of Histological Observations.

From the serial biopsies, it may be possible to reconstruct the events occuring as tissue destruction progressed. Mild villus edema was the first indication of tissue damage. With increased villus edema, there was progressive elevation of the mucosal epithelium from the villus surface. Goblet cells became depleted of mucous. Then the villus tips, distended by edema, sloughed off. Mucosal epithelial cells lining the crypts seemed to be preserved best, and usually were the last area of mucosal destruction.

There was usually only moderate edema of the submucosa, but often marked perivascular edema in the muscularis. Generally, the muscularis was well preserved, and only in the 24 hour perfusions was necrosis observed.

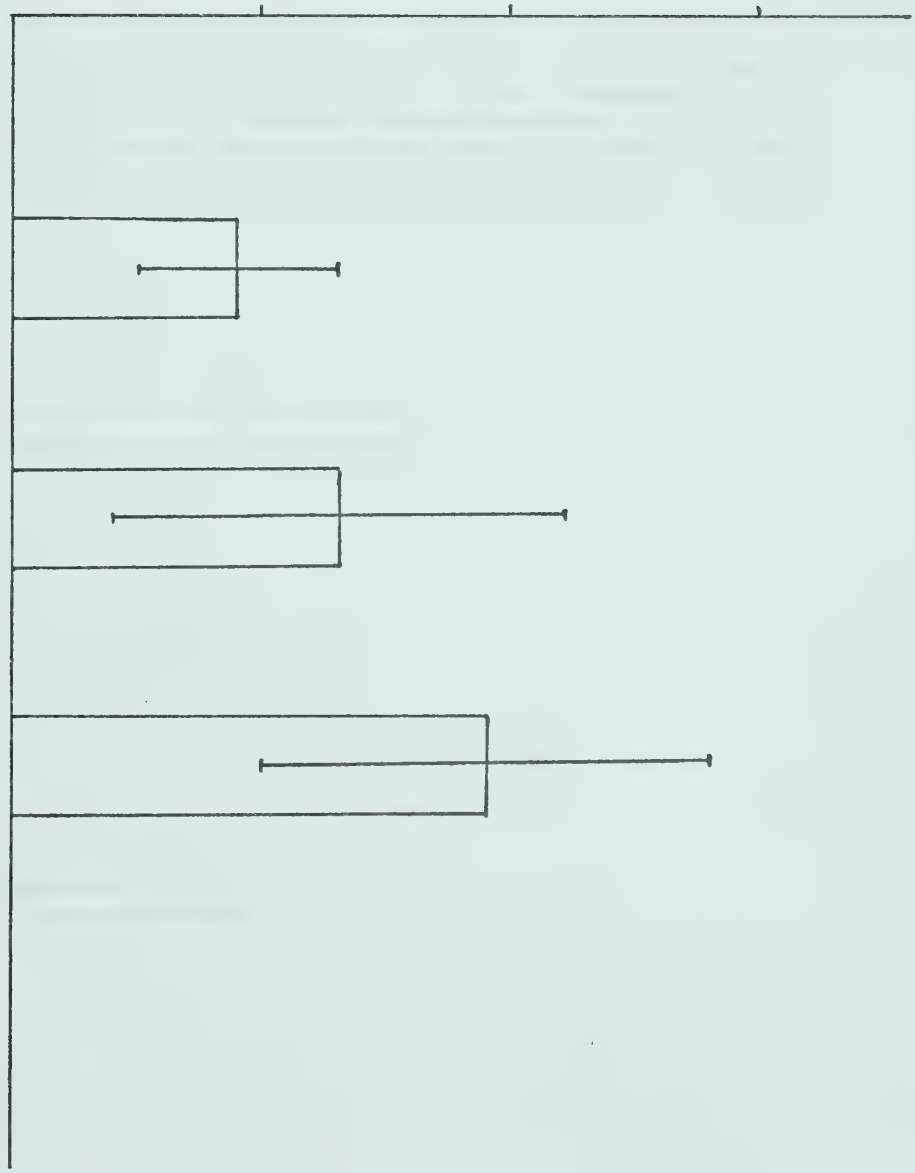
In segments preserved four and eight hours, the addition of chlorpromazine to oxygenated perfusate was associated with less

edema and better cellular preservation.

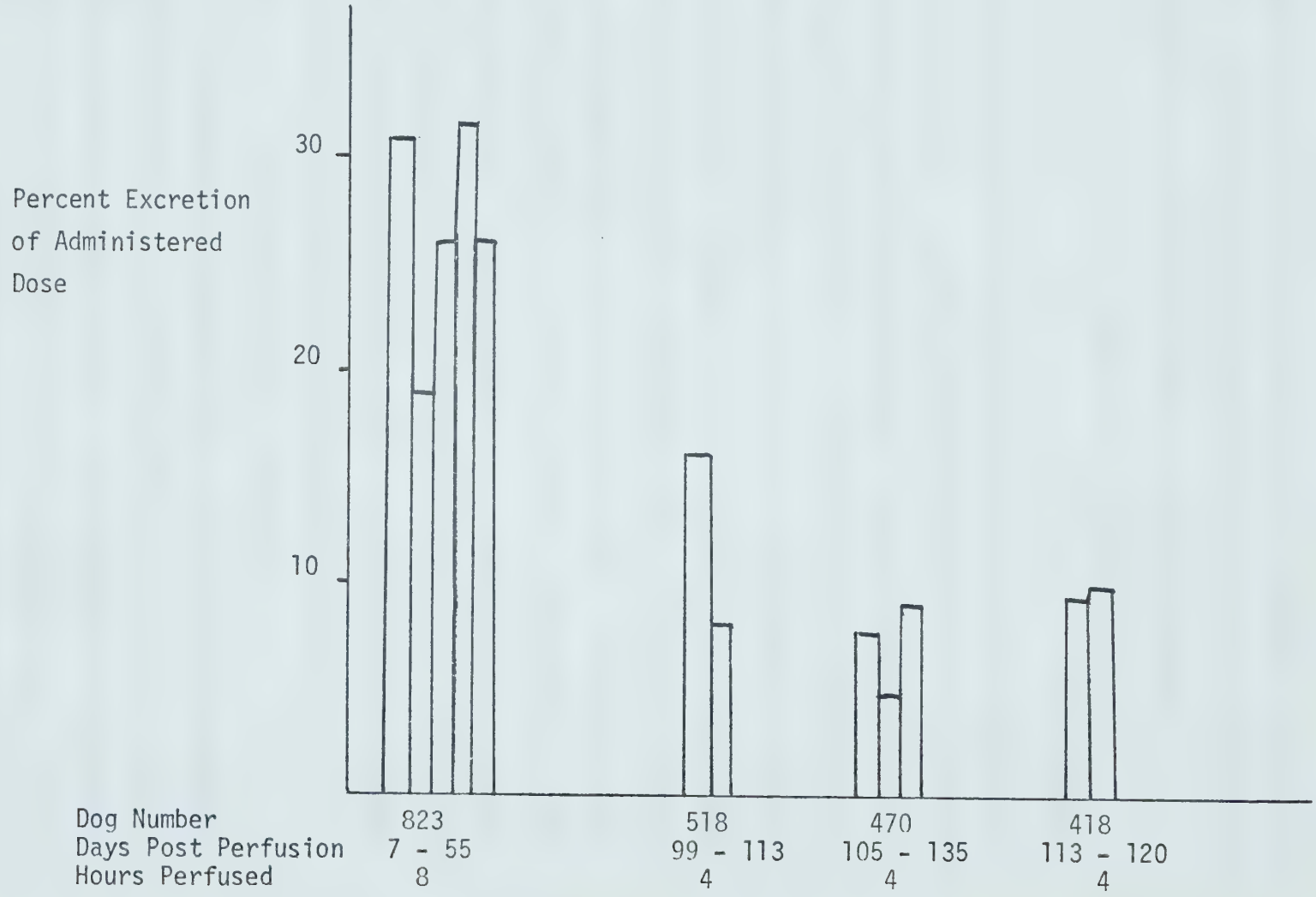
F. Vitamin B_{12} Absorption.

All perfused segments successfully reimplanted absorbed vitamin B_{12}. Absorption of segments preserved four hours was similar to that of controls; absorption of the eight hour group was statistically different from that of controls. Dogs which had several tests carried out showed uniform vitamin B_{12} absorption from one test to another. See Graphs three and four.

Graph 3. Vitamin B_{12} Absorption



Graph 4. Multiple Vitamin B_{12} Absorption Tests



10. Discussion.

A. Evolution of Preservation Method.

The project was begun using techniques proven to give successful renal preservations. It was found that segments of intestine responded to these methods differently than the kidney, and several modifications in the procedure were necessary.

1. Intralumenal Fluid Accumulation.

In the first experiments, the segments of intestine were heaped upon themselves on a rack in the perfusion chamber. Fluid accumulated in the lumen to such an extent that intestinal distention caused the perfusion pressure to rise. The most obvious cause was obstructed venous and lymphatic drainage. An attempt to correct this by suspending the bowel from the chamber walls failed. Therefore, a perforated tube was threaded through the lumen of the bowel and the fluid was allowed to drain continuously. This did not correct the initial problem, but prevented the bowel from becoming turgid, and allowed perfusion pressure to remain low.

Reintroduction of this fluid into the perfusion circuit appeared to be toxic to the preserved segments. Therefore, frequent additions of fresh perfusate were necessary to maintain the circulating volume.

2. Poor Mucosal Preservation.

Uniform survival of segments perfused for four hours was possible without the use of an oxygenator in the circuit. Survival of segments perfused eight hours was not obtained without

an oxygenator. Because of the possibility that the amount of oxygen present was insufficient despite suppression of cellular metabolism by hypothermia, perfusate PO_2 was increased. The addition of a membrane oxygenator increased both perfusate PO_2 and tissue oxygen consumption, but did not increase survival post-reimplantation. Chlorpromazine was then emperically added to the perfusate because it had been reported to result in better preservation of intestine in hypothermic-hyperbaric preservations (101, 102). The combination of an oxygenator in the circuit plus chlorpromazine gave better intestinal preservation and post re-implantation survival.

It was observed that the temperature of the preserved bowel rose rapidly while the vascular anastomosis was being constructed. Further anoxic damage probably occurred during this period. To maintain hypothermic temperatures, the bowel was placed on a cold mat prepared by freezing a laparotomy sponge previously soaked in saline. By this means the temperature was kept at about 10°C until the termination of the anastomosis.

3. Vascular Anastomosis.

Characteristic of the failed autoimplanted segments in the initial experiments were thrombi adherent to the suture lines of the vascular anastomoses. Initially this was attributed to lack of surgical skill (104), but as the technique became more meticulous, the thrombi continued to occur with annoying frequency. Heparin was employed, first in full anticoagulating doses and

then, to avoid hemorrhagic complications, in partial doses. The heparin had no value in itself, but it helped to uncover the real source of trouble - white thrombi.

Bathing the vascular anastomotic site with an isotonic solution of magnesium sulfate, while intermittantly pinching the site to disperse clumps of platelets, prevented vascular occlusion for only short periods. The problem was next attacked by pre-medications aimed at inhibiting platelet agglutination. The combination of acetylsalicylic acid, prednisone and dipyridamole had an inconsistent effect in preventing thrombosis. Finally the combination of partial heparinization and the platelet inhibitors was used and thrombosis more regularly prevented.

B. Surgical Preparation.

The major factor in organ damage was most likely trauma inflicted by the perfusion procedure. However, manipulation of the intestinal segment during excision and reimplantation may have caused additional tissue damage. For instance, thrombosis may have been caused by operative trauma to vascular endothelium. Clamping, cutting, flushing blood from the vessels, and re-anastomosis of the blood vessels may have damaged the endothelium about the site of vascular anastomosis.

Proximal vessel stumps ligated for 24 hours (group 6) may have incurred anoxic endothelial damage which predisposed them to thrombosis when blood flow returned.

In some instances, lymph nodes accompanying the vascular pedicle may have compressed blood vessels near the vascular anastomosis, causing turbulence and thus thrombosis.

Lymphatic obstruction caused by the dissection may have contributed to interstitial edema because of poor tissue lymph drainage. This factor would be important in edema of both the perfused and reimplanted segments.

C. Modalities of Perfusion.

1. Temperature.

Hypothermia was an important factor in the preservation of isolated intestinal segments. In this experiment, hypothermic perfusion resulted in intestinal survival beyond the known limits of normothermic ischemic viability.

The optimal temperature for intestinal preservation is unknown. Hypothermic temperatures have been employed in successful intestinal preservations - 2°C in hypothermic-hyperbaric preservations, and 9°C in perfusion preservations (appendix one and two). Temperatures about 10°C are frequently used in renal preservations (23,28).

On the other hand, intestine might be better preserved at a higher temperature, a compromise being sought between better control of flow due to maintenance of autoregulation, and reduced metabolism (76).

2. Flow Rate and Vascular Resistance.

Flow rates used to perfuse intestinal segments were within the reported normal range of intestinal flow in the intact animal (52). By co-relating the oxygen demands of hypothermic intestine with the oxygen solubility in hypothermic plasma, it was found that the flow rate employed could provide adequate tissue oxygenation. This flow rate (approximately 50 cc per minute) also permitted adequate recycling of the perfusate to maintain the organs at the desired temperature.

Systolic and diastolic pressures recorded in this experiment were within the range reported in observations for canine In Situ distal mesenteric arterial pressures (68,94). The pump did not exactly duplicate pulsations produced by a normal In Situ heart. The frequency of pulsation was 16 to 20 beats per minute, and because of inherent characteristics of the roller pump, systole was unusually prolonged. The resultant abnormally high or prolonged pressures may have contributed to tissue edema by raising the capillary pressure and increasing fluid filtration.

The vascular resistance observed in the hypothermic perfused intestine was higher than that reported from studies of normothermic In Situ bowel (1). There are several possible causes for the persisting increased vascular resistance in this experiment. Increased perfusate viscosity (7), decreased vascular distensibility (17), and edema (4), are considered to be the most likely

factors involved. Increased resistance would tend to reduce flow in the perfused intestine, and thus may have contributed to poor tissue preservation.

Observed progressive reduction in vascular resistance was unlike that reported in other studies of isolated organs, and is difficult to explain. A drop in vascular resistance is said to occur within thirty minutes and then remain at a constant low value (4,7,21,23). Perfusate loss from the circuit as edema or intestinal lumenal fluid did not occur at a rate sufficient to account for a marked drop in resistance. Possibly deterioration of the blood vessels in the perfused segment was associated with a gradual increase in vascular distensibility.

The increase in tissue edema throughout these perfusions was not associated with increasing vascular resistance. This is in keeping with claims that an organ must be encapsulated in order to have flow affected by the hydrostatic pressure of edema (71,72). Therefore, observation of pressure changes during perfusion of intestine does not appear to have the same value in predicting the eventual reimplantation success as it does in the kidney (17,23).

3. Perfusate.

The perfusate used in these experiments was emperically constituted and probably did not fulfill all physiologic requirements. One possible source of damage was the low perfusate concentration of calcium. Hypocalcemic solutions have been

associated with loosening of intercellular bonding (62), and may have been partly responsible for the mucosal epithelial slough.

Cellular and interstitial edema may have been aggravated by the perfusate. The osmolarity, and sodium and potassium concentrations represented normal extracellular values. In the system of preservation used, hypothermic inactivation of the sodium pump may have been associated with edema. This edema might have been prevented by the use of a perfusate of intracellular electrolyte composition (56,57).

Availability of nutrients in the plasma perfusate was not studied in detail in this project. The variety and quantity of nutrients in fresh plasma was assumed to be sufficient for the hypothermic intestine. Addition of glucose and insulin to the perfusate was done empirically because it had been shown to benefit renal hypothermic preservations (28).

Cortisone and chlorpromazine were added to the perfusate because they have been shown to give better tissue preservation with other organs (33, 32, 102, 106). Two noticeable effects of the addition of chlorpromazine to the perfusate were the reduction in oxygen consumption and better tissue preservation. Better tissue preservation, most obvious in reduced mucosal epithelial sloughing, less interstitial edema, and better mucosal epithelial cell preservation, might be the result of chlorpromazine-membrane stabilization (101). Reduction of mucosal epithelial intracellular

edema may account for better mucosal cell preservation. Stabilization of vascular endothelial cell membranes might preserve endothelial function with less extravasation of perfusate.

Because it was possible to perfuse the segments at relatively low flow rates and pressures, it was assumed that the viscosity of the perfusate was not a problem in this experiment. Therefore the viscosity of the perfusate was not intentionally adjusted.

One final possible detrimental effect of the perfusate was embolic vascular obstruction resulting from denatured lipoproteins. In this experiment, prior cryoprecipitation, and filtration of the plasma during the perfusion seems to have prevented this complication.

Loss of perfusate into the intestinal lumen may have contributed to tissue damage. Sodium and potassium concentration, and the osmolarity of the lumenal fluid was identical to that of the perfusate. Slight change of the perfusate may have been harmful to the perfused organs.

Increased vascular permeability coupled with sloughed mucosal epithelium may have allowed absorption of toxic materials from intestinal lumen. Histamine, serotonin, bacteria or bacterial cell wall components may have been absorbed and circulated through the perfused intestine, contributing to tissue damage.

D. Tissue Preservation.

i. Lumenal Fluid.

Accumulation of fluid in the lumen of the perfused intestine has been reported to occur during most perfusions of normothermic intestine (appendix I.). In this experiment, fluid with the same concentration of sodium and potassium as the perfusate, was observed to accumulate in the lumen throughout the perfusion.

One cause may have been the normally high fluid permeability of the villus capillaries allowing transudation of perfusate into the bowel lumen (68,71). Hypothermic inactivation of enzymes may have prevented active reabsorption of extravasated fluid. In addition, drainage of edema via lymphatics may have been reduced due to operative trauma, or absent peristalsis. Accumulating edema in the villi could then result in fluid loss into the intestinal lumen. High perfusion pressure or hypocalcemic perfusate might result in disruption of villus capillaries, allowing perfusate extravasation. Disruption of intestinal villi by increased interstitial hydrostatic pressure would allow loss of perfusate directly from torn capillary ends.

Accumulation of intestinal fluid may be a sign of poor tissue preservation, particularly of the delicate intestinal villus and it's capillaries. If allowed to collect, it may so distend the intestine that vascular obstruction occurs. In this study,

continuous drainage of lumenal fluid was instituted to avoid these complications.

ii. Oxygen Consumption

The calculated intestinal oxygen consumption at 10°C is about 0.001 cc/gm/minute (144). In this experiment, the oxygen consumption approximated this value when no oxygenator was used (0.00075 cc/gm/minute).

Increasing the perfusate PO_2 with an oxygenator was associated with increased oxygen extraction. The possibility that the apparent increase in oxygen consumption was merely oxygen lost by diffusion into the organ chamber was not investigated. However, because the chamber was closed, diffusion of oxygen from the segment would tend to equilibrate, and diminish continued loss.

The ability of the perfused segments to extract greater amounts of oxygen when the PO_2 was elevated suggests that in the hypothermic intestine normal PO_2 tensions are insufficient to insure that all cells are well oxygenated. Additional support for this concept may be derived from the observation that oxygen consumption was reduced after the addition of chlorpromazine to the perfusate. Increased oxygen made available to the organ may be thought to benefit preservation. However, this theory was not verified by better histological preservation of tissue when an oxygenator was added to the circuit. Chlorpromazine, when added

to the oxygenated perfusate, reduced the oxygen consumption almost 125%. The combination of the use of an oxygenator with chlorpromazine was associated with better tissue preservation, and supports the findings of Eyal (101).

iii. Thrombus Formation.

1. Platelet Thrombi.

Platelet thrombi were observed to occur at the arterial anastomotic site in vessels where there was no apparent local obstruction. Such thrombi were not observed in other areas of the reimplanted segments. Generation of these thrombi was probably due to exposed vascular collagen or locally released ADP or thrombin.

The silk suture used in the vascular anastomoses was occasionally seen to protrude into the vascular lumen and to be encompassed by the platelet thrombus. Possibly the suture material generated platelet agglutination, or carried collagen into the vascular lumen which in turn stimulated thrombosis.

It is interesting that vascular thrombosis did not become a major problem until the attempted reimplantation of segments preserved for eight hours. This suggests that tissue damage was progressive. Reasons for vascular damage may be similar to those attributed to poor mucosal preservation (see discussion of mucosal preservation).

2. Mixed Thrombi

Mixed thrombi occurred in the vessels of the autografted segments chiefly in response to vascular obstruction, at site of

possible turbulence, or in association with histologically demonstrated marked tissue destruction (mainly in groups three and six). Compression or twisting of the perfused organ's vascular pedicle could produce blood stasis initiating clotting (106).

Mixed thrombi occurring in association with poor tissue preservation may be explained in several ways. Disruption of blood vessels within the perfused intestine exposed the circulating blood to foreign surfaces, and tissue thromboplastin initiating coagulation. Thrombi starting at local sites may then propagate along vascular channels.

Sluggish vasomotor control in poorly preserved segments allows pooling of blood after reimplantation. Hypoxia and acidosis then might have generated local clot formation. Hypotension of the host animal due to hemorrhage or loss of fluid into the lumen of the graft can result in reduced perfusion and further acceleration of clot formation.

3. Anticoagulation.

Anticoagulation was helpful but not always successful in preventing thrombosis in the autograft. There was reduction in incidence of white thrombi from 100% in the three instances of group five where the anticoagulation regimen was not used, to 50% in anticoagulated group five subjects.

The anticoagulation regimen had no effect where there was a major stimulus to thrombus formation such as vascular compression

or marked tissue destruction.

Bathing the vascular anastomotic site with dilute magnesium sulfate did not prevent platelet thrombi, possibly because the vessel walls were too thick for adequate absorption of the solution.

Hemorrhage was an associated problem with the use of anticoagulants. The success of a vascular anastomosis depends in part on temporary limited thrombus formation at the site of the joined vessel ends (104). Uncontrolled bleeding particularly at arterial anastomoses contributed to the failure of several reimplantations.

iv. Mucosal Preservation.

The perfusion technique used in these experiments did not answer all the requirements of intestinal preservation. There was progressive deterioration of tissue as perfusion was prolonged, in spite of attempts to make the modalities of perfusion as physiologic as possible.

Edema seemed to play an important role in mucosal epithelial slough, and villus disruption. Intestinal weight gain during perfusion was observed to be due to the accumulation of intracellular and interstitial fluid. Inactivation of cellular membranes by hypothermia or an adequate perfusate probably initiated edema. Progressive edema may have several explanations. The normal high intestinal capillary filtration rate (68,71),

unmodified by autoregulation (68,72,137) could account for the marked interstitial edema. Lack of a pressurized organ chamber may have aggravated this fluid loss by producing a relatively increased intravascular hydrostatic pressure. Poor lymphatic drainage due to surgical obstruction or absent intestinal peristalsis during perfusion may have contributed to accumulation of edema. Interstitial edema could then lead to cellular separation and slough.

Damage to the organ may have causes other than the perfusion techniques. Surgical manipulation and the periods of warm ischemia no doubt contributed to some tissue destruction.

Continued mucosal epithelial and villus tip slough occurred for the first 24 hours after reimplantation of the perfused segments. This may have been due to irreversible damage to the organ during perfusion, or to the effects of the reimplanted organ on the host. Tissue hypoxia or continuing vascular thrombosis in the reimplanted segment seem to be prominent possible causes of further tissue deterioration.

The reimplanted bowel may have been deleterious to the host. Continued loss of fluid into the lumen of the reimplanted intestine may have made the host animal hypovolemic. Decreased perfusion of blood to the reimplanted bowel would promote its deterioration. Toxic material may have been absorbed from the intestinal lumen of the graft, impairing the host's health, and secondarily leading to degeneration of the graft.

v. Reimplantation.

When the vascular clamps were released, the reimplanted segment immediately regained normal color, implying no gross vascular obstruction was caused by the perfusion. The absence of hyperemia suggests that vasomotor tone was rapidly regained, at least in vessels observed near the serosal surface.

The early appearance of peristalsis demonstrated the functional preservation of myocytes in the muscularis. Hyperperistalsis observed in segments preserved eight hours might be due to anoxia, acidosis, transmembrane electrolyte imbalance or reactivation of accumulated neurohumoral transmitting substances (138). Loss of peristalsis several hours after reimplantation of the 24 hour segments suggests that the observed hyperperistalsis was due to poor muscle cell preservation. This was supported by the observation of post perfusion myocyte necrosis.

Increased perivascular edema after reimplantation may have been the result of poor capillary preservation or vascular disruption during perfusion. Lymphatic obstruction may also have contributed to the edema. However, resolution of the edema within several hours suggests that damage to either capillaries or lymphatics was quickly reversible.

vi. Gross Observations.

Adhesions occurring after reimplantation were an important sequelae. The cause of adhesions is not clear. Possibly they

resulted from exudation of plasma on the serosal surfaces of the perfused segment, which then organized into fibrotic bands.

Prevention of obstruction due to adhesions might be possible by carefully suturing the bowel to itself or to the peritoneum, to prevent random adhesions and kinking.

The possibility of adhesions complicates the clinical applications of intestinal preservation. Although absorptive functions might remain intact, kinking of the perfused segment could result in intestinal obstruction which would reverse the clinical benefit of transplanted intestines.

vii. Vitamin B_{12} Absorption.

There is no apparent reason for the increased absorption of vitamin B_{12} from segments preserved for eight hours. Possibly there were too few animals in the series for adequate statistical evaluation.

The results of the vitamin B_{12} absorption testing was useful to the experiment because it demonstrated that despite prolonged perfusion, cells remained capable of either carrying on their function, or of regenerating other functional cells.

11. Summary.

Modification of the original experimental protocol made it possible to successfully autograft segments of ileum preserved for up to eight hours. Absorptive capacity as measured by vitamin B_{12} absorption was retained. Better tissue preservation was found to occur with the use of an oxygenator, and the addition of chlorpromazine to the perfusate. Successful reimplantation of the segments was found to require a regimen of anticoagulation.

Several problems were left unsolved. One of these was poor preservation of intestinal mucosa in perfusions of longer duration than eight hours. A second problem was the continuous loss of perfusate into the intestinal lumen during perfusion. One final problem arising from the experiment was the occurrence of adhesions in the reimplanted perfused segments, complicating the long term function of the bowel. Each of these problems requires solution before transplantation of perfusion-preserved intestines can become a clinical reality.

It is encouraging that in spite of the apparent damage to the mucosa, it was able to rapidly regenerate. Thus, in the space of a week, the histological picture of a segment of intestine which had been markedly damaged, reverted to a normal appearance. This attests both to the fragility of the mucosal epithelium and villi, and to the resistance of the mucosal cells of the crypts. Hopefully by altering some of the parameters of perfusion, the period of preservation could be extended.

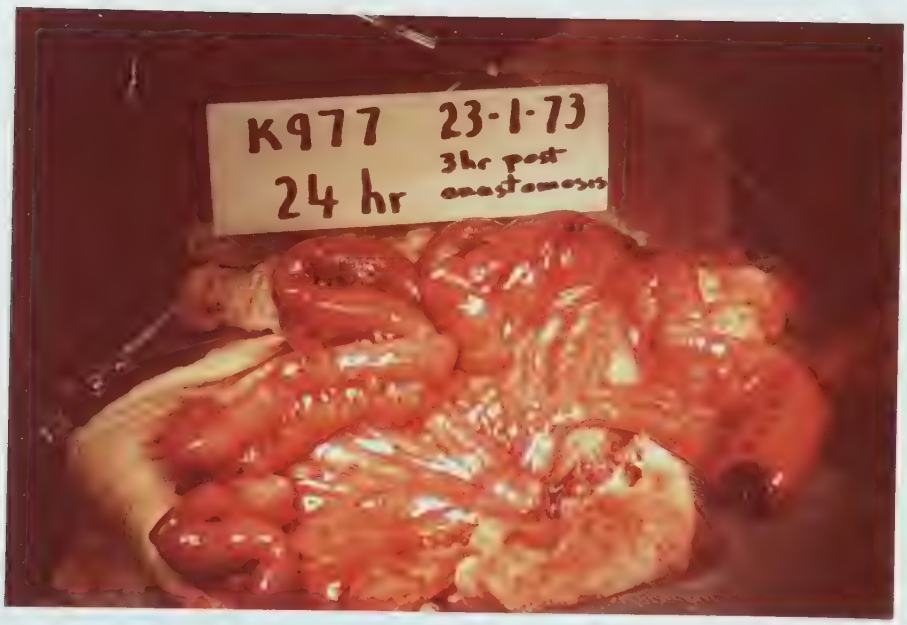
Illustration 2. Perfusion apparatus housing a perfused segment of intestine. See Illustration 1 for labels.

Illustration 3. Intestine in perfusion chamber. Suspension hooks, tube draining lumen, and arterial cannula are visible.

Illustration 4. Intestine distended by intralumenal fluid when lumen was not constantly drained.

Illustration 5. Intestine perfused 24 hours and autografted. Peristalsis is evident. Mesenteric edema and hemorrhages, and mucosal hemorrhages are marked.


Dec 13/72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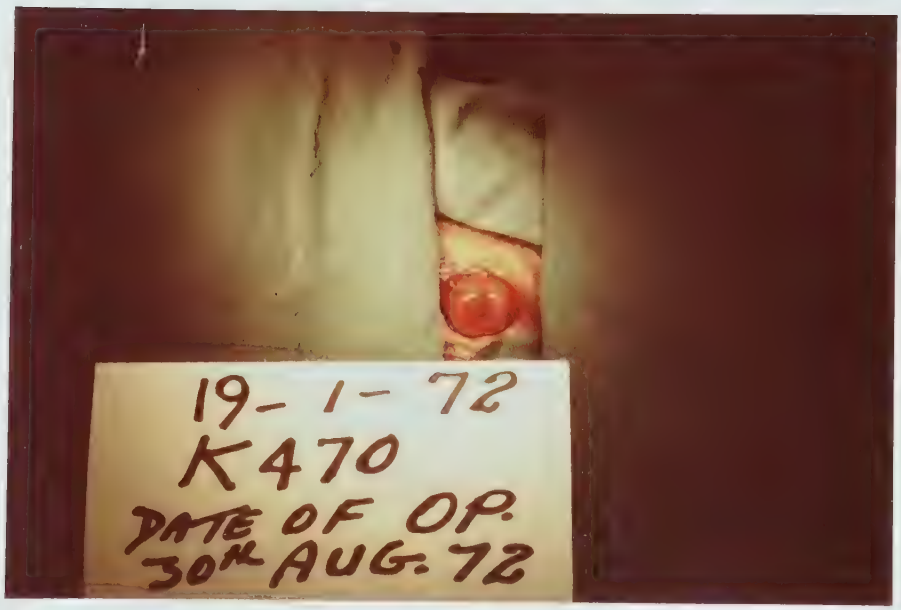
24 hr
3hr post anastomosis

Illustration 6. Stoma of autografted intestine, perfused eight hours. Mucosa color is normal. Stomal discharge is slightly sanguinous.

Illustration 7. Intestine perfused four hours, 142 days after autografting. Stomal mucosa is well maintained.


1090
Feb 19-73
8 hr.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30th AUG. 72

Illustration 8. Intestine perfused four hours, 142 days after perfusion. At laparotomy the mesentary is thickened and there are adhesions involving the preserved intestine.

Illustration 9. Intestine perfused eight hours, one day after autografting. Venous thrombosis caused the hemorrhage into the intestinal wall.


ORMAL
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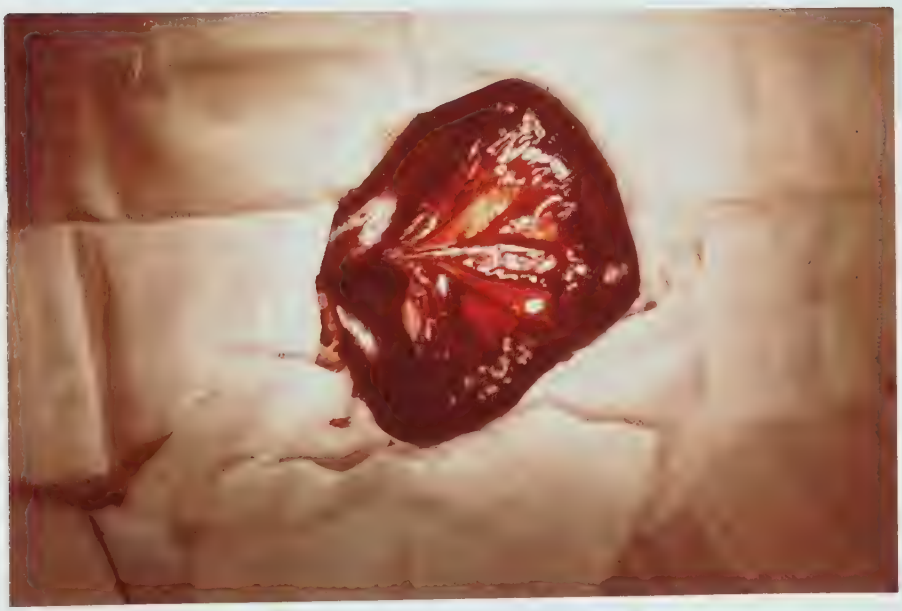
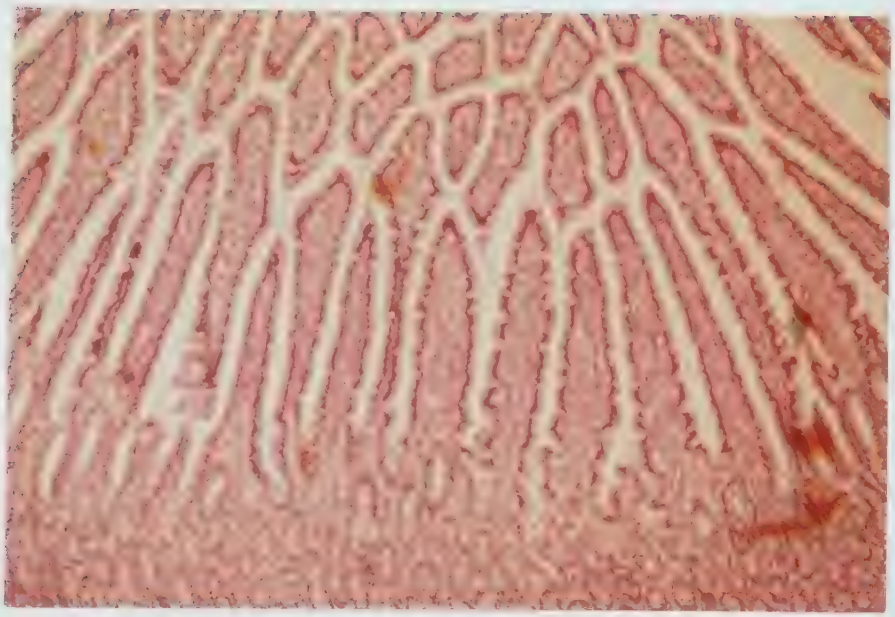


Illustration 10. Micrograph of normal ileum mucosa.

Illustration 11. Micrograph of segment preserved four hours, when no oxygenator or chlorpromazine was used. Section taken 20 minutes after autografting, shows edema of muscularis and submucosa, hemorrhagic areas in the mucosa, loss of villus epithelium and preservation of the epithelial cells of the crypts.



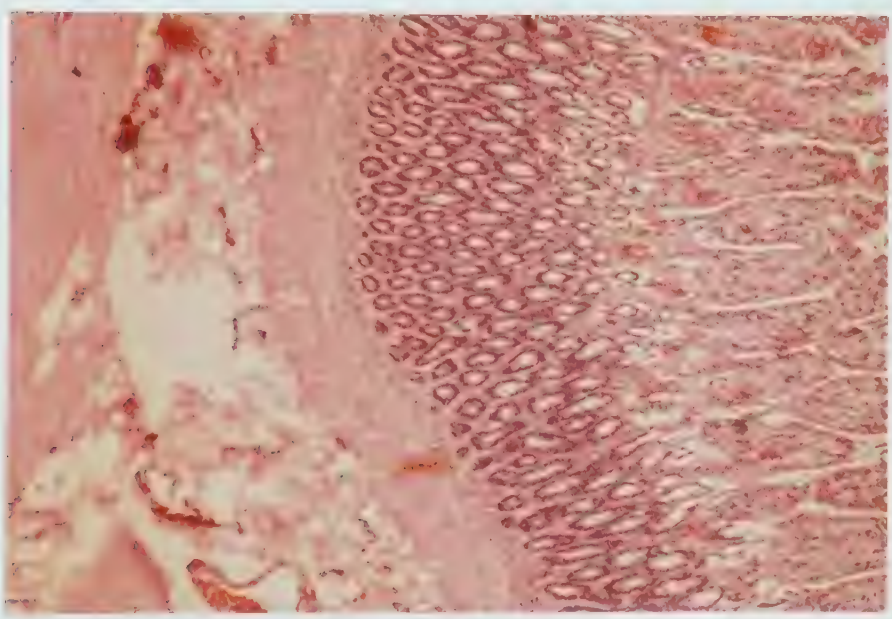
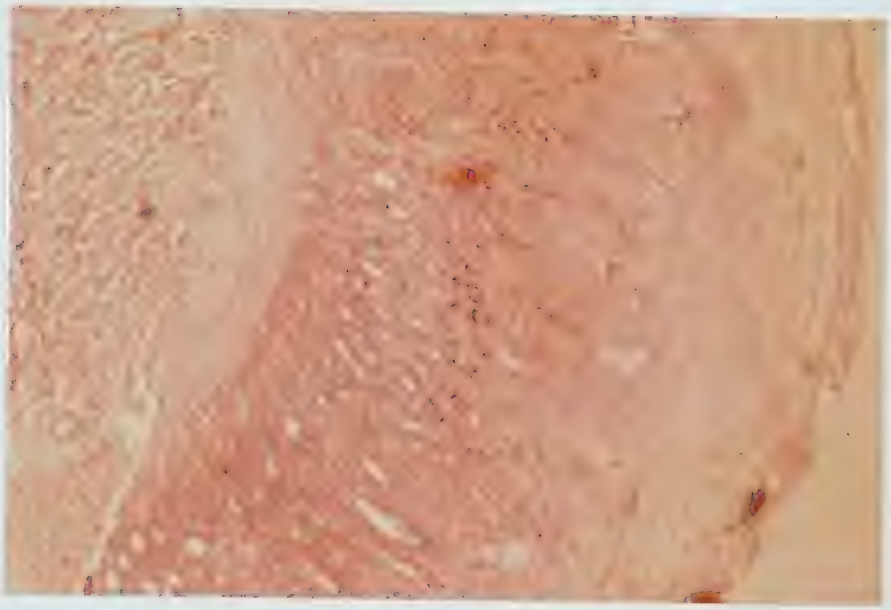


Illustration 12. Micrograph of same segment shown in illustration 11, taken two days after autografting. It shows normal muscularis, hemorrhagic areas in the submucosa, preservation of the epithelial cells of the crypts, but loss of the villus architecture.

Illustration 13. Micrograph of the same segment shown in illustration 11, taken 55 days after autografting. The intestinal mucosa has a normal appearance.



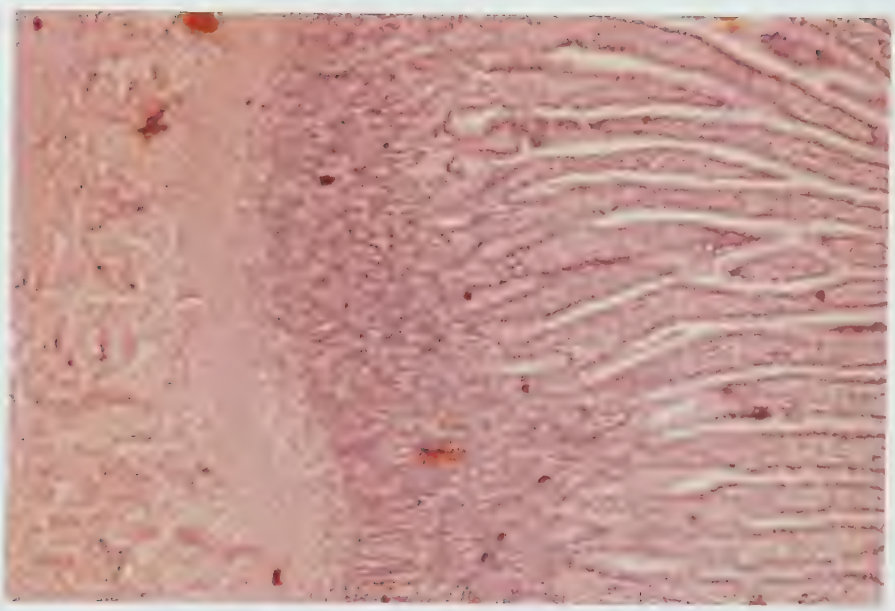
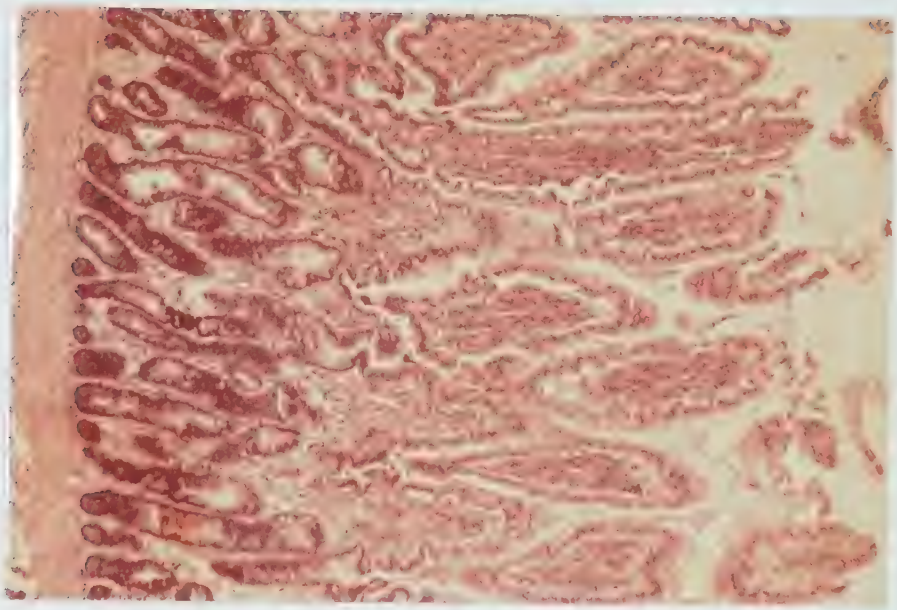


Illustration 14. Micrograph of segment perfused eight hours using oxygenator and chlorpromazine. It shows better mucosal preservation than the four hour segment depicted in illustration 11.

Illustration 15. Micrograph of segment after 24 hours perfusion using an oxygenator and chlorpromazine. It shows destruction of the villi and edema of the basal mucosa and submucosa.



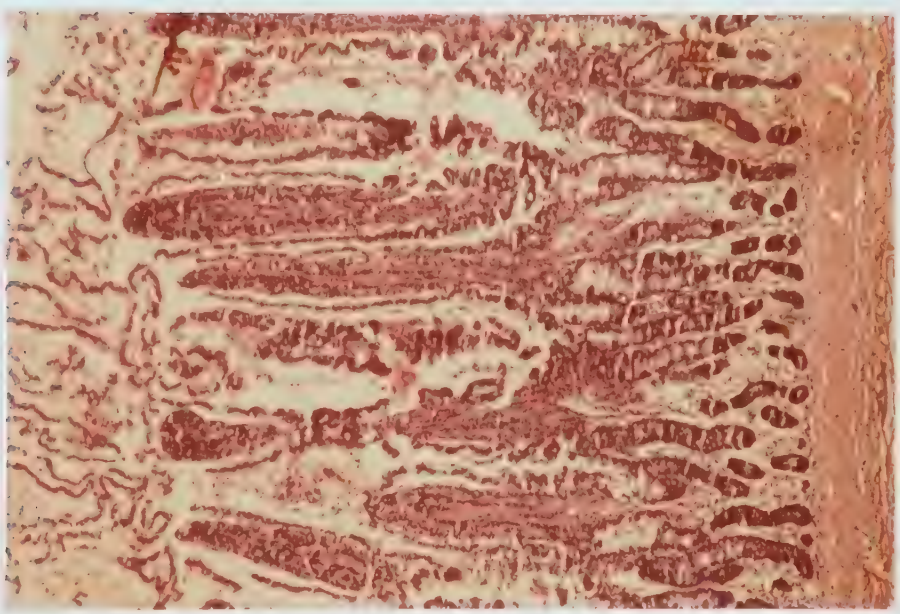
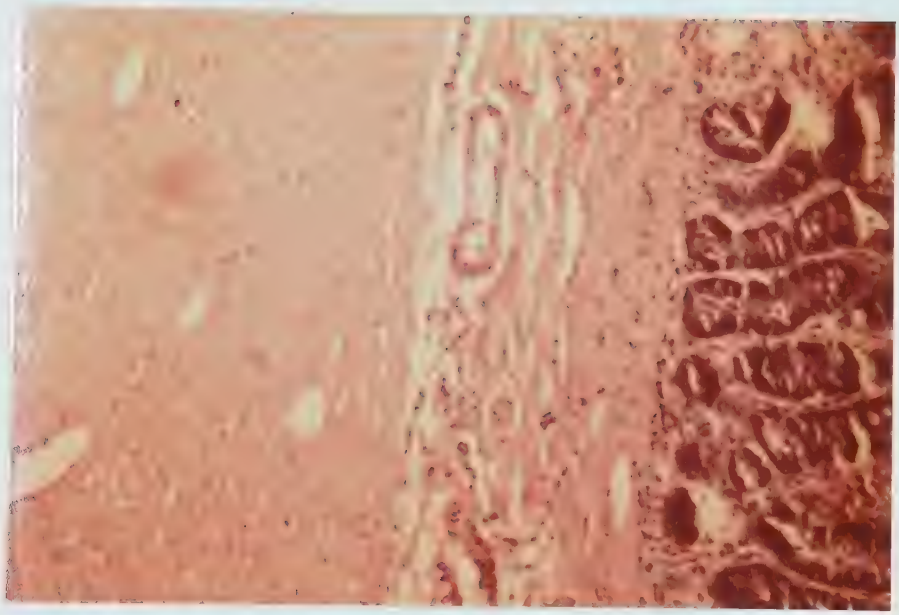
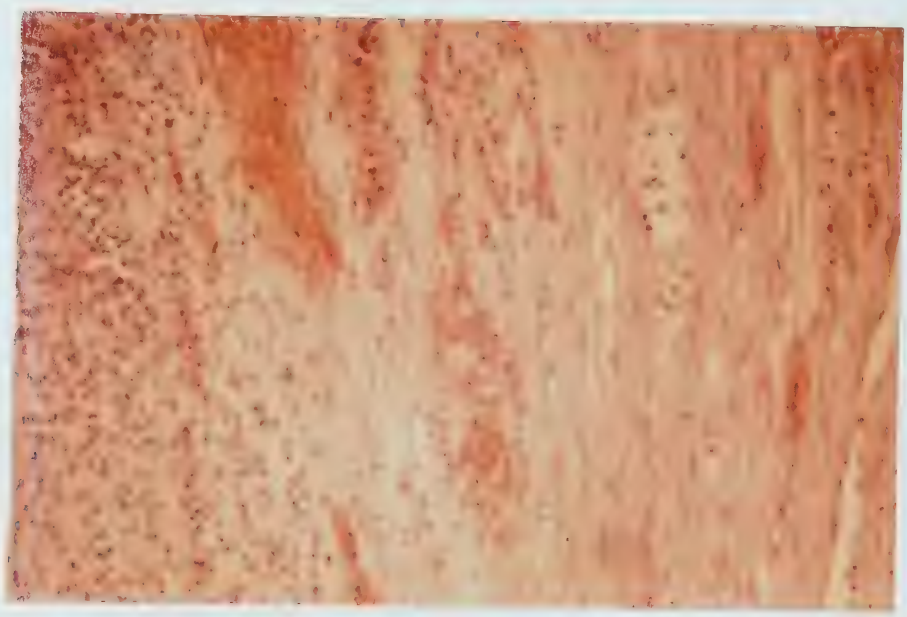


Illustration 16. Higher magnification of segment shown in Illustration 15. It shows edema of the muscularis and submucosa, and necrosis of the epithelial cells of the crypts.

Illustration 17. Micrograph of segment shown in illustration 15, taken 24 hours after autografting. The mucosa had completely sloughed and there are hemorrhagic areas in the muscularis.





APPENDIX I - INTESTINAL PERFUSION-PRESERVATION LITERATURE REVIEW.

REFERENCE	AUTHOR	HOURS PERFUSED	ORGAN	QUALITY FLOW	TEMPERATURE °C
93	Salerno	5	dog sigmoid	pulsatile	38
94	Iijima	5	whole dog small bowel	pulsatile	38
84	Austen	18	whole dog bowel	non-pulsatile pulsatile	38
2	Alican	24	whole dog small bowel	pulsatile	9
76	Windmueller	5	whole rat small bowel	pulsatile	38
113	Kavin		whole rat small bowel	pulsatile	38
52	Hohenleitner	5	dog small bowel	pulsatile	38
139	Ruiz	2	dog ileum	pulsatile	39
130 131	Lojacono Zuccetti	3	dog ileum	pulsatile	37

PRESSURE mm/Hg	FLOW RATE cc/min	PERFUSATE	LUMENAL FLUID	EDEMA	MOTILITY DURING PERFUSION
110-140 / 50-70	12	blood/ electrolyte	3.2cc/hr mucus	yes in 5 hours	YES
---	120-150	blood/ electrolyte	"normal"	YES	YES
140-160	90	whole blood	75-100 cc/min	marked	diminishing
---	150-200	Homologous Serum	---	NO	YES
95	0.5/gm	whole blood	much isosmotic with plasma	---	hypermotility
---	1-2/gm	whole blood	Isosmotic	YES	YES
80-110	76-114/ 100 gm	blood/ electrolyte	0.8-2.4 cc/min/ 100 gm	---	YES
70-80 / 30-40	.34-.44 gm	blood/ electrolyte	---	5% wt. gain	to stimulation
---	---	blood/ electrolyte	---	---	---

HISTOLOGY	IMPLANTATION SUCCESS	ABSORPTION
Submucosal Edema	None	----
Edema	Yes	Uptake of tritiated Thymidine in tissue culture after preservation
---	---	---
---	---	---
---	None	---
Normal	---	Glucose & H_2O absorption during perfusion
---	---	---
---	---	glucose absorbed In Vitro
Normal	---	d-xylose absorbed In Vitro
---	Yes	---

APPENDIX 2 HYPOTHERMIC-HYPERBARIC INTESTINAL PRESERVATION LITERATURE REVIEW

REFERENCE	AUTHOR	TEMP °C	O_2 PRESS. ATA	HOURS DURATION	IMPLANTATION SUCCESS	MUCOSAL MICRO TEST OF VIABILITY
140, 141	Lillehei	2	7.9	24	YES	slough
33	Manax	2	3	24	YES	slough
		2	-	5	NO	
		-	3 7 9	5	NO	
127	Manax	2	3	24	YES	normal
		2	3	48	NO	necrosis
		2	8	24	YES	normal
		2	8	48	YES	normal
		2	15	72	YES	normal
75	Lyons	4	7.9	24	YES	--- glucose, chloride
		4	7.9	48	YES	--- and H_2O abs.
101	Eyal	4	---	24	NO	slough
		4	CPZ	24	YES	slough
		4	c+CPZ	72	YES	slough
144	Rudolf	2	3	24	YES	slough glucose abs. in 1 yr.
143	Mandel	2	3	24	YES	normal, then
		2	8	24	YES	slough at 3 days then normal at 2 wks.
142	Mormose	4	7.9	72	NO	slough uptake tritiated
		4	3	72	YES	normal thymidine
		4	1	72	YES	normal
		4	3	96	NO	-----
145	Idezuki	4	3	22	YES	

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